

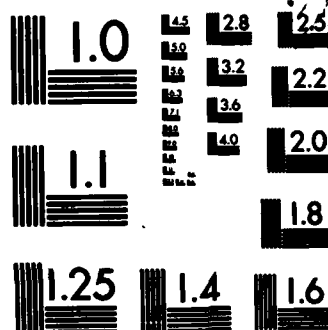
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ABSTRACTS



OCEANIC BIOLOGY PROGRAM

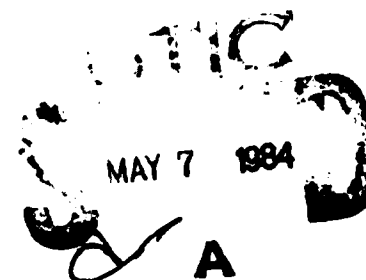
Oceanic Chemistry and Biology Group

Office of Naval Research



DECEMBER 1982

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The 1982 Progress Report Abstracts of the Oceanic Biology Program represents the fourteenth year in which the projects supported by the program have been represented in a report of this nature. Progress Report Abstracts is the single document of the Program that brings together all the investigations receiving support and relates them to broad naval problem areas while presenting a brief of the scientific objectives and accomplishments. The purpose of these Abstracts is twofold. They serve to introduce and relate | | |

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the Program to other offices and bureaus of the Navy as well as other government agencies. Secondly, but perhaps more importantly, they serve as an avenue for coordination and communication among investigators whose projects are sponsored by this Program. A broad range of studies in marine biology and biological oceanography is represented. Techniques and approaches used by one discipline may also prove useful to others, and the forum presented by these abstracts can provide an interchange that normally might not result from a literature search or dialogues with colleagues.

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The abstracts are preliminary in nature and do not constitute publication in the conventional sense. They are considered PRIVILEGED PERSONAL COMMUNICATIONS and must not be referred to without the written consent of the respective investigator and then only as a personal communication. For the most part, the investigations referred to will be published at a later date and will become part of the scientific literature.

Credit for this volume belongs entirely to the investigators who conducted the research and wrote the abstracts. We appreciate their cooperation and take pride in the high quality of research that ONR has the privilege to sponsor.

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BIOLOGICAL SIGNAL/NOISE PROGRAM

ACOUSTICAL

ACOUSTICAL TECHNIQUES FOR THE STUDY OF NEKTON AND ZOOPLANKTON AT WATER TYPE
BOUNDARIES AND OCEAN FRONTS

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N00014-79-C-0703

083-503

OBJECTIVES

To study nekton and macro zooplankton at the interface between different types of water. Studies indicate that volitional behavior is involved in the distribution of animals across the interface. Since the distributions are space and time dependent, we need rapid means of determining fish densities across the interface. Experience shows that acoustics is a powerful tool and that the poor resolution of common fisheries type sonars is inadequate. Objectives: Year 1: (a) To build an additional receiving system for our Simrad 120 kHz sonar. The new receiver is to have high resolution multibeam capability. (b) To build real time acoustic data processing systems. Year 2: To use our high sonar systems to study mysis, alewife, and smelt at fronts on Lake Michigan or Lake Superior. Years 3 and 4: To take our high resolution systems to the ocean and measure fish densities at the interfaces between water types.

ABSTRACT

The first phase of our research was (1) to develop instrumentation to process fish echoes digitally, (2) to build and operate a multibeam high resolution sonar, (3) to do mid-water trawling, and (4) to improve our theoretical and analytical techniques for interpretation of fish echo data. The second phase was to make field studies with our systems at an oceanic front.

This is a preliminary report of our cruise to the boundary of the Gulf Stream east of Cape Hatteras during July. Briefly stated our (APPLE II) computer controlled data acquisition and processing systems worked. The multibeam sonar worked. The mid-water trawl caught mid-water "critters" and fish. There were differences from one side of the front to the other. Everybody has data and specimens. We expect a fruitful winter of data processing and paper writing.

We show two examples of our acoustic results. Figure 1 shows the plot of integral echo squared data. The sonar echoes were processed by analogue to digital converter and dedicated squaring preprocessor under control of an APPLE II. The data are for 1 meter depth increments beneath the transducer. The data show relative maximum concentrations of scatters at two depths.

The frequency function of echo amplitudes at the output of the transducer is proportional to the convolution of the probability density function (PDF) of the directional response of the transducer and the PDF of the scattering process at the fish $w_F(e)$. We have devised a simple numerical procedure to remove (deconvolve) the transducer effects. This gives $w_F(e)$ and fish density N_F . An example for alewife (*Alosa Pseudoharengus*) in Lake Michigan is shown in Figure 2. The curve "N" is assumed to be noise, "A" is assumed to be alewife, and "U" is an unknown larger fish. The alewife are 10-12 cm long and the length of the larger fish is estimated to be about 40 cm.

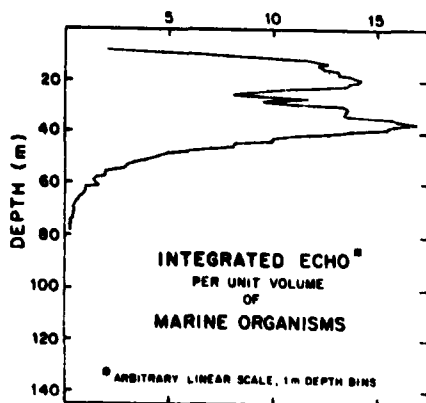


Figure 1. Relative biomass vs. depth.

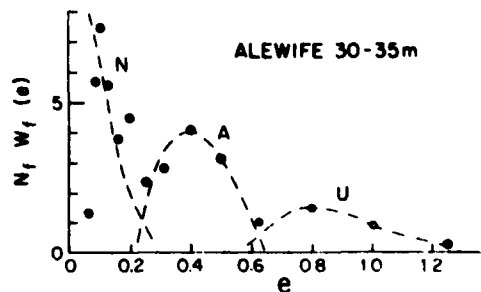


Figure 2. Amplitude frequency of fish echoes. The units of e are volts (v) and the units of $N_F W_F(e)$ are $10^{-4} (m^{-3}v^{-1})$.

PLANS FOR THE FUTURE

The task for the winter is to integrate the biological, oceanographic, and acoustic results. We need to do some sonar instrumentation to overcome deficiencies of our commercial sonar equipment. We need additional software to improve real time displays of acoustic data. We are planning for a summer cruise.

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STUDIES OF SMALL WHALES (GRAMPUS GRISEUS AND GLOBICEPHALA MACRORHYNCHUS)
MOVEMENT PATTERNS USING SATELLITE AND ACOUSTIC TOWED ARRAY TECHNOLOGY

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N00014-82-C-0298

NR 104-157

OBJECTIVES

The major objective of this study is to apply recently available miniaturized satellite transmitter (Nimbus-6 satellite system and ARGOS) and towed acoustic array technologies in the study of long-term movement patterns of small odontocete cetaceans.

ABSTRACT

Our previous project "Biology of Small Whales", started in 1977, provided a reasonable data base on distribution and relative abundance of pilot whales (Globicephala) and Risso's dolphins (Grampus griseus) in the Southern California Bight as a function of water temperature, season and food abundance. The quarterly aerial and ship surveys of the study area are continuing. Because of past success, the towed acoustic array system continues to be a major component in all ship surveys. The specific emphasis of the current project is to document the dynamics of what appears to be a relationship between the previously mentioned temporal and oceanographic factors and the seasonal movement patterns of the two species of small whales being studied. In addition to acoustic/visual survey methods, these patterns will be determined by instrumenting one or more pilot whales and/or Risso's dolphins with a miniaturized satellite radio transmitter. The WHOI/OAR VHF whale radio tag (modified) will be used as a backup system. During 1982 the following tasks have been completed.

1. NMFS dolphin transmitter designed to work with the Nimbus-6 system has been refurbished, modified for operation in Southern California Bight and calibrated.
2. Attachment system, saddle and release mechanism for use on pilot whales and Risso's dolphins has been designed, fabricated and tested.
3. The above attachment system has been modified to also work with the compact ARGOS PTT Wisco model 165 transmitter terminal and the WHOI/OAR VHF whale transmitter.

4. Arrangements have been made and equipment procured to collect corollary sea surface temperature and water color data from Nimbus-7 CZCS satellite imagery captured by NASA and the SIO Remote Sensing Facility concurrent with the planned whale tracking.
5. With the cooperation of the NMFS, the towed array was deployed from the NOAA ship David Starr Jordan on a transect from Manzanillo, Mexico to Hawaii. A major purpose of this cruise was to calibrate cetacean visual censusing techniques. The towed acoustic array system was also calibrated.

PLANS FOR THE FUTURE

A major effort will be made during the fourth quarter of 1982 and first quarter of 1983 to instrument and track a pilot whale and/or Risso's dolphin with the available satellite transmitters. In cooperation with NMFS a 30-40 acoustic/visual census of the Southern California Bight is scheduled for May 1983.

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- Thomas, J. A., S. R. Fisher and L. M. Fern. 1982. "Preliminary results on marine mammal detection using a towed acoustic array in the Eastern Tropical Pacific". HSWRI Tech. Rept. 82-144.
- White, M. J., J. G. Jennings, W. F. Gandy and L. H. Cornell. 1981. "An evaluation of tagging, marking and tattooing techniques for small delphinids". NOAA TM-NMFS-SWFC-16. 142 pp.

PREPARATION OF A LONGHURST-HARDY PLANKTON RECORDER

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N00014-80-C-0440

NR 083-005

OBJECTIVES

(a) To modify and test a Longhurst-Hardy Plankton Recorder (LHPR) built in 1974 and last used in 1977, (b) to use the improved LHPR in studies of zooplankton patchiness and vertical distribution in the eastern Pacific Ocean.

ABSTRACT

The Longhurst-Hardy Plankton Recorder developed at Woods Hole Oceanographic Institution by Haury and Wiebe in 1974 has been extensively modified to improve its operational characteristics and potential to measure a variety of oceanographic parameters simultaneously with the collection of sequential zooplankton samples. An entirely new electronic system to control the LHPR and acquire and record data has been built, tested and used routinely in the field. The new electronics utilize a 12-bit microprocessor which controls LHPR function. The system in its present configuration measures temperature and depth; additional sensors planned are salinity, chlorophyll fluorescence, and possibly light. Control of the LHPR is achieved from a new deck unit, used either alone or in conjunction with a Commodore 8032 microcomputer for preliminary data processing and storage on floppy disk and printer. The LHPR can be operated independently of conducting cable, deck unit, and microcomputer by presetting tow parameters in the underwater unit; data in this mode is recorded on a Sea Data cassette tape recorder in the underwater unit.

The newly modified LHPR has been used on a recent cruise (SOSO, 30 July - 30 August 1982, R/V New Horizon) from Honolulu to San Diego to investigate (a) the vertical structure of zooplankton communities in various oceanographic regimes and (b) the relationship of this vertical structure to the vertical distributions of chlorophyll a and primary productivity. Twenty oblique LHPR tows were obtained from depths as great as 650 m to the surface; each tow consisted of from 30 to 70 individual samples. No problems were encountered with the operation of the new electronics and data acquisition/processing systems.

PLANS FOR FUTURE

(a) To procure additional sensors and data processing/display capabilities for the LHPR, as well as to obtain spare electronics boards for the deck and underwater units, (b) to test the LHPR in conjunction with the Shulenberger, Lange and Johnson towed video system, and (c) to conduct a joint investigation with the video system of the relationships between fine- to mesoscale distributions of zooplankton and physical features and processes such as fronts, eddies, Langmuir circulation, and internal waves.

CURRENT REPORTS AND PUBLICATIONS

Haury, L. R. and P. H. Wiebe. 1982. "Fine-scale multi-species aggregations of oceanic zooplankton". Deep-Sea Res., 29, pp 915-921.

BIOLOGICAL SENSORS FOR THE DETECTION OF
ELECTRIC, MAGNETIC, AND HYDRODYNAMIC FIELDS

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N00014-80-C-0440

NR 083-005

OBJECTIVES

(a) To determine the imposed and self-induced electric, magnetic, and hydrodynamic fields that marine organisms detect, to reveal the biological information the organisms derive from these fields, and to analyze the pertinent sensory systems, (b) to contribute to such Naval issues as electromagnetic detectability, object retrieval, guidance and navigation systems, shark bite, corrosion, drag reduction, and environmental effects, (c) to provide a thorough treatise of the results in a text on electric and magnetic field detection.

ABSTRACT

Elasmobranch fishes have specific receptors for the detection of dc and low-frequency electric fields in the nanovolt/cm range. Naturally occurring fields inform the animals about the positions of their prey, the drift of ocean currents, and the magnetic compass directions. Magnetic bacteria have ordered arrays of single-domain magnetite crystals that passively align the cells with the earth's magnetic field. These organisms offer excellent objects for a systems analysis of the ferrimagnetic compass mechanism. Marine fishes and invertebrates have a variety of hydrodynamic sensors that show great potential for the detection of prey and inanimate objects. These sensors may also serve in a feedback system to control locomotion and to reduce drag.

After establishing the magnetic compass sense of Urolophus halleri, it remained to be determined whether the stingrays indeed rely on the self-induced electric fields as was anticipated from the theory of electromagnetic orientation. Therefore, the fish were deprived of the earth's magnetic field and tested on their responses to imposed electric fields simulating those they would receive in the presence of the earth's magnetic field. This research, however, has been troubled by a faulty new power supply (replacing the one that was damaged by lightning). Moreover, it soon became evident that swimming distances were too short in the 6-ft test tanks to provide the rays with the proper sensory information. Thus, it was decided first to upgrade the electromagnetic setup that served the experiments for which it had been designed so well, before continuing these crucial, and up to the this stage promising, tests. This decision fortuitously coincided with the project's move from Woods Hole back to La Jolla.

In the meantime, we have been testing the catfish Plotosus anguillaris on its sensitivity to uniform electric fields to determine whether this secondarily marine teleost has fully adjusted its electroreceptors to the much lower voltage gradients of the oceanic milieu (together with Dr. Obara in Japan).

The electric fields that elasmobranch fishes detect have been plotted 3-dimensionally on the project's microcomputer. The spatial aspects were enhanced by having the observer move about in the picture. Until now, work has focused on the fields used to simulate prey. Plots of self-induced and ocean-current fields will follow. This research not only gives great insight into the configurations of the various electric fields, but also makes it possible to determine the spatial and temporal characteristics of the stimuli that the fishes receive. This is precisely the information needed for a biologically meaningful systems analysis of the animals' electric sense. A thorough understanding of the naturally occurring and man-made electric fields is also fundamental to the evaluation of electrically evoked shark bite. Above all, these studies have set the stage for the development of a new, non-destructive corrosion monitoring system.

For the work on the hydrodynamics of lateral-line detection, a displacement measurement/reproduction system has been designed. The system was tested on the fields of calmly swimming prey fish as sensed by a predator from striking distance. Fourier analysis was carried out by a newly written microcomputer program. As expected, the amplitudes of the lower-frequency components (3 Hz and less) were by far the largest. Detailed information of this kind is of utmost importance to break the deadlock in the study of hydrodynamic detection in aquatic animals. At Scripps, experiments have been continued together with my former collaborator in the Netherlands, Dr. Peter Goerner. By recording from the lateral-line nerves, we are investigating the response characteristics of Platyrrhinoidis triseriata to hydrodynamic stimulation within the range of dc to 200 Hz. In concurrent behavioral tests, we are observing the feeding responses of Urolophus halleri to hydrodynamic stimuli in the same low-frequency range.

PLANS FOR THE FUTURE

(a) To complete the studies on the electromagnetic nature of the elasmobranchs' magnetic compass sense, (b) to verify the fishes' use of electroreception in orientation at sea, (c) to conclude the current statistical-mechanics studies on magnetic bacteria, (d) to measure and represent 3-dimensionally the electric fields relevant to electroreception, shark bite, and corrosion, and (e) to conduct analogous studies on hydrodynamic-field detection.

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BIOLOGICAL AND ACOUSTICAL STUDIES OF MIDWATER ANIMALS

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N0014-79-C-0004

NR083-102

OBJECTIVES

Our objectives are (1) to describe the variability in the horizontal and vertical distributions and abundances of oceanic micronekton, and (2) to evaluate and improve acoustical and net techniques for quantitative assessment of micronekton.

ABSTRACT

Our present program involves the use of towed, multi-frequency acoustical measurements and opening-closing midwater trawls to estimate the distributional patterns of mesopelagic animals. We are especially interested in meso-scale (1-50 km) variations in the composition and distribution of mesopelagic micronekton, physical and biological correlates with these patterns, and the processes that produce them.

Broadband data from our 1981 "Arcer" cruise are being analyzed to estimate the size distribution and abundance of gas bubbles associated with migratory and non-migratory scattering layers off Oregon. Preliminary results indicate good agreement between the relative abundance of the different gas bubble radii from the arcer estimates and from the swimbladders of fishes caught in trawls at the same depths. Scattering strengths measured at discrete frequencies on this cruise are significantly correlated with the total biomass of midwater trawl catches at the same depth, but the scattering per unit biomass was an order of magnitude higher during the day than at night because of the higher proportion of fishes with gas-filled swimbladders found during the day.

We are developing a net sonar system to measure the escape speeds, reaction distances and trajectories of animals in the tow path of a net. A cruise to test this system is scheduled for September 1982 in Monterey Bay, CA.

During August 1982 we completed a "Patterns" cruise to study variations in the mesoscale distribution in the scattering intensity, abundance and composition of scattering layer organisms. A distinct, high-intensity patch was localized and measured acoustically for the several days that it persisted. Midwater trawls, CTD's, fluorescence, irradiance and zooplankton tows were made.

We have completed a study of the zoogeography of mesopelagic fishes of the subarctic Pacific based on 641 midwater trawl collections. Two basic distributional patterns were found: species associated with subarctic and species associated with transitional-waters. Several variations of each of these patterns were apparent.

PLANS FOR FUTURE

- 1) Complete our analyses of the broad-band and discrete frequency data from the "Arce" cruise, and the discrete frequency and biological data from the "Patterns" cruise.
- 2) Publish our study on the zoogeography of mesopelagic fishes.

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Stein, D. L. and W. G. Pearcy. "Aspects of reproduction, early life history, and biology of macrourid fishes off Oregon, U.S.A." Deep-Sea Res., in press.

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STUDIES OF OCEAN VOLUME REVERBERATION
AT HIGH ACOUSTIC FREQUENCIES

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N00014-81-C-0562

104-965 and 104-977

OBJECTIVES

(1) To improve our capability to measure and understand zooplankton distribution and dynamics by applying ultra-high frequency acoustic technology to the problem of measuring absolute zooplankton abundance by size classes, (b) to interrelate the acoustically derived information on zooplankton distributions to temperature structure and chlorophyll distributions.

ABSTRACT

We have determined that small zooplankton, e.g. copepods as small as 0.1 mm, can be detected using ultra-high frequency acoustics (up to 10 MHz). The quantitative analysis of the acoustic scattering has revealed complex structure in both the horizontal and vertical dimensions. These measurements have been correlated with variability in zooplankton collected with a high volume pump at the same time and place as the acoustic measurements. Acoustically directed sampling of millimeter size zooplankton can now be routinely accomplished. Associations of the acoustically detected zooplankton structure with temperature structure, chlorophyll profiles, anchovy larvae and the depth distribution and thickness of fish schools have also been noted.

Results of our work include the the assessment of several scattering models which relate observed acoustic scattering and the scattering projected on the basis of zooplankton collected by a plankton pump. Regressions relating abundance and size of the collected zooplankton, through scattering models, to observed scattering have confirmed our hypothesis that the dominant scatterers in our experiments have been zooplankton. Positive correlations between volume scattering strengths and plankton volumes were also noted (r^2 for the regressions varied from 0.63 to 0.84). Achieving an in-depth understanding of this process and its parametric sensitivities was essential before approaching the more difficult inverse problem--deriving zooplankton size-distributions from multi-frequency acoustic measurements.

We have also measured the target strength of individual zooplankters at a range of frequencies from 100 kHz to 10 MHz. Preliminary assessments of this data are in agreement with the scattering models presently being used. About 75% of these echoes have been processed.

We have also continued work on the inverse problem as it applies to our attempts to extract zooplankton size from acoustic scattering data for multiple frequencies. A rigorous mathematical proof of the uniqueness of the solution of this problem was completed and reported.

Progress in the development of our updated measurement and data acquisition systems has occurred in different areas. We have increased the number of frequencies from four to 21 frequencies (100 kHz to 10 MHz), have designed and tested transmitters and receivers, and have written the software for the new data logging system. We have further tested our surplus underwater vehicle and have modified the control systems to provide better towed vehicle response.

PLANS FOR THE FUTURE

During the current year we are concentrating our efforts on (a) further testing the second-generation data-acquisition and measurement system (21 acoustic frequencies, ancillary sensors, shipboard data gathering and storage), (b) analysis and processing of the target strength data from individual organisms, and (c) analysis and preparation for publication of results from prior cruises. Plans include operation of the second-generation system to (a) extract size and abundance information on the zooplanktonic scatterers and (b) to utilize this system in various study areas to determine the interrelationships between the zooplankton distributions and distributions of temperature, salinity, and chlorophyll.

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ACOUSTIC TRANSDUCTION IN FISH

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OBJECTIVES

(a) To examine signal analysis by the ear of teleost fishes through physiological recordings from single neurons of the eighth nerve, (b) to test the hypothesis that the fish ear is capable of doing a spatial analysis of signals in a fashion analagous to the 'place' mechanism of the mammalian ear, (c) to determine the acoustic roles of different otolithic organs through analysis of the responses of each organ to sounds, and (d) to determine inter-specific differences in the structure of the ear and examine the correlations between these structural differences and any variations in inner ear function.

ABSTRACT

Earlier investigators had suggested that the whole saccule (the otolith organ of the fish ear most often associated with sound detection) responds to each acoustic signal and that the only function of the saccule is for the transduction of acoustic stimuli. However, recent studies, using morphological, physiological and behavioral techniques have lead to the suggestion that the ear is likely to perform complex signal analysis including determination of the direction of a sound source. Studies of hair cell orientation patterns in the saccule, as well as physiological recordings from the ear and analysis of the neural innervation patterns of the various otolith organs support such an idea, although we have yet to directly prove the existance of such a mechanism or its specific role(s) in sound detection and signal analysis.

One of the major hypotheses to come from recent work has been that the saccule is potentially involved in a spatial analysis of signals in such a fashion that different signals may maximally affect different regions of the sensory epithelium - thereby providing a possible functional analogue to the well-known place mechanism of the mammalian ear for spatial analysis of sounds along the length of the cochlea. Studies in other laboratories, recently repeated by ourselves, have used intense tonal stimulation to study ear responses. Preliminary data suggest that different regions of the saccule (and possibly the lagena) are maximally affected by different frequencies, although the linear tonotopy found along the length of the mammalian cochlea for different frequencies does not appear to exist in the teleost saccule. We have also demonstrated that the innervation of the saccule by the saccular branch of the eighth nerve is reasonably complex. Different regions of the saccule are innervated by separate neurons, with little overlap of receptive fields on the saccule by different neurons. Thus, it is possible that the responses of different

saccular regions (presumably responding to different signals) are carried separately to the central nervous system for further processing.

PLANS FOR FUTURE

(a) To further test the hypothesis for spatial analysis of signals within the fish ear using morphometric, behavioral and physiological techniques, (b) to determine the nature of signal analysis (e.g. is it frequency, intensity, and/or directional analysis), (c) to test for spatial analysis in each of the other otolith organs, (d) to determine inter-specific differences in spatial analysis in species with marked differences in inner ear structures, and (e) to examine the function of the teleost ear through mathematical modeling.

CURRENT REPORTS AND PUBLICATIONS

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COGNITIVE CHARACTERISTICS OF MARINE MAMMALS

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OBJECTIVES

(a) To study the cognitive characteristics of sea lions in the context of training sea lions to comprehend strings of words constructed in artificial gestural and verbal languages, (b) to compare the cognitive characteristics of sea lions with the cognitive characteristics of dolphins.

ABSTRACT

At our laboratory in Hayward and at Marine World/Africa, U.S.A., we are training and testing two California sea lions (a six-year old female, Rocky, and a three-year old male, Bucky) with an artificial language consisting of gestural signs produced by movements of a trainer's arms and hands, as well as, human speech signals. The present results encompass ten months of training for Bucky and fourteen months of training for Rocky. During the first phase, the male acquired four actions ("fetch," "flipper-touch," "tail-touch," and "toss"), and the female acquired three actions ("fetch," "flipper-touch," and "mouth") under control of the trainer's gestures. During the second phase of training, Rocky and Bucky demonstrated that they could immediately extend these actions, which were originally directed to a small white ball or a tether ball, to most any floating or sunken novel object. In the third phase, two objects (a large yellow ball and white pipe for Rocky and a red baseball bat and white ring for Bucky) were mapped onto additional gestural signs. As was the case with action signs (verbs), the assignment of object symbols (nouns) was arbitrary.

During the next tutoring phase, novel objects were introduced to both sea lions without special training, i.e., they were mapped directly on to novel gestural signs combined with action signs. Thus far, when there have been at least two alternative objects available, Rocky has responded entirely correctly (i.e., to the correct object with appropriate action) to 38 out of 40 novel two-word commands, and Bucky has responded entirely correctly to 27 of 30 novel two-word commands. In most cases, new nouns were best understood by Rocky and Bucky in the context of earlier learned nouns rather than later learned nouns. Currently, Rocky shows complete mastery of 40 object-action combinations (8 object names and 5 actions), and Bucky shows comprehension of 35 object-action combinations (7 object names and 5 actions).

Do these gestural signals translate from symbolic representation of objects to mental images of objects in sea lions? Evidence from an ongoing study, the "miss-

ing object" experiment, suggest they do. In this experiment rather than two objects being present on any given trial, five, six or seven objects are present at one time on the surface of the water and are constantly changing position because of wave and wind action. When Rocky is given a symbol for a missing object, she spends up to 10 seconds from a stationary position searching for the object. She indicates her search is over by repositioning herself for receiving the action signal. When given the signal, she usually "refuses" to perform the signaled action (verb). On the other hand, if the object is present she immediately (within two seconds) orients to it following the symbol presentation, awaits the action signal and when released, performs appropriately better than 95% of the time. Thus, it appears that sea lions can be taught to understand that symbols represent classes of external objects without support from contextual cues and have the ability to translate these symbols into temporally dynamic searching images allowing for appropriate behavioral adjustment.

PLANS FOR FUTURE

Currently, both sea lions have been trained to station in front of the trainer/teacher, attend to all gestural and speech signals and withhold their reactions to objects until they are released by a tactile signal. This procedure will enable us to perform a myriad of future experiments on the working memory of California sea lions, including associative memory, serial memory, storage capacity retrieval cueing and displacement. In addition, we plan to continue extending the vocabulary of both sea lions, incorporating new language classes such as modifiers (small, large, dark, light, go left, go right, go below, go above) and ultimately moving on to three, four, and five-word sentences made up of modifier-direct object-modifier-indirect object.

In order to evaluate the informative value of signals to the sea lions, we must accurately describe the animal's actions in response to the trainer's signals. Such an enterprise will require permanent video documentation of many aspects of training and testing.

CURRENT REPORTS AND PUBLICATIONS

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ECOLOGICAL ENERGETICS OF DEEP SCATTERING LAYER ANIMALS:
IN SITU STUDIES

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OBJECTIVES

(a) To determine the in situ metabolism of dominant migrating and non-migrating DSL animals at depths of maximum concentrations, (b) to determine the day and night behavior of dominant DSL animals by direct observations, (c) to determine the energetic importance of gas floats and swimbladders in the vertical migrations of mesopelagic siphonophores and fishes, (d) to determine the in situ volumes of siphonophore gas floats and fish swimbladders and ascertain their acoustic scattering properties.

ABSTRACT

In situ metabolic measurements of the dominant DSL animals, the siphonophore Nanomia sp. and the larval fish, Sebastolobus altivelis, were made using the slurp gun respirometer (SGR) with Alvin (March 1982) in the Santa Catalina Basin. We identified two distinct deep scattering layers in the Santa Catalina Basin, one at 400m and one centered at 600m. The shallower DSL was composed primarily of physonect siphonophores (Nanomia sp.), a suborder characterized by having gas floats. The DSL at 600m was composed predominantly of myctophids and larval fishes of the genus Sebastolobus (having swimbladders).

Our sampling efforts were concentrated on the 400m DSL in collection of 7 individual animals of the genus, Nanomia sp. These animals were gently captured with the SGR and their oxygen consumption and nitrogenous excretion rates continuously measured while being incubated at 400m on a mooring line for 28 to 48 hours. In addition 2 individual larval fishes of the genus, Sebastolobus altivelis, demersal fish as adults (Moser, 1974), were captured similarly at 600m with the SGR and their in situ metabolism measured for 48 hours. cursory examination of the data reveal definite diurnal pattern in respiration rates of both the siphonophore, Nanomia bijuga and the larval fish, Sebastolobus altivelis, with increased rates occurring at night. A similar diurnal fluctuation in respiration was found in the midwater fish, Cyclothone acclinidens, another dominant DSL fish. Behavioral observations reveal that the dominant animal in the 400m DSL, Nanomia sp., actively swims and feeds throughout the period of observation from 1000 to 1700. Myctophid fishes, (including Stenobrachius leucopsaurus and Triphoturus mexicanus) were dominant in the 600m DSL, and assumed various orientations from horizontal to vertical in both upward and downward looking positions. These fishes

generally appear in a state of lethargy but occasionally undergo quick bursts of speed in any direction which appears to be initiated either by the presence of prey or predator. In contrast to this general myctophid behavior, Sebastolobus sp. appear to actively swim during the daylight periods of observations even when not noticeably disturbed by the submersible with low light intensity lights.

PLANS FOR FUTURE

(a) To determine the energetic importance of gas floats and swimbladders in the vertical migrations of mesopelagic siphonophores and fishes, (b) to determine the in situ volumes of siphonophore gas floats and fish swimbladders and ascertain their acoustic scattering properties.

CURRENT REPORTS AND PUBLICATIONS

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MARINE BIOLOGICAL SOUNDS

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OBJECTIVES

The environmental biology of marine mammals, particularly of cetaceans, with emphasis on underwater acoustic behavior, effects of noise, interaction between animals, and the movements of individuals and populations.

ABSTRACT

A deep-water cruise in the southeastern Caribbean demonstrated nearly ideal conditions for study of local cetaceans, particularly sperm whales. This population was surveyed acoustically as well as visually, and good underwater recordings were made which included sequences of the repetitive sound patterns, or "codas", which appear to serve as individual acoustic identifiers.

Beginning tests of 360° scanning sonar (50-60 kHz) to track whales underwater succeeded in tracking finback, humpback, minke, and right whales. Target strengths differed, depending upon species and aspect ratios.

Correlations of the low frequency sounds (17 to 125 Hz) of finback whales with their activities, confirmed previous associations and documented the relationships. Analyses of these sounds continue, including an extensive data set of finback sounds recorded by unsuspecting WHOI geo-physicists near the mid-Atlantic fracture zone.

Base-line studies of species in the Cape Cod area continue to provide new information, as well as the background of experience for comparison with other work. The pattern of whales alternating at the surface was photographed as three right whales took turns at the surface, each on 15-min. intervals. This points out the need for identification of individual whales for realistic assessments of distribution and abundance.

Analysis of the surfacing data from the radio tagged finback whale in the Denmark Strait confirmed diurnal changes in dive times and swimming speeds. Over the entire 9 1/2 day, 2000 km track, the whale's speed averaged 4.8 kts., averaging 1.98 min. between surfacings.

Sonar studies and radio tagging of humpback whales in Glacier Bay, Alaska, was undertaken in cooperation with the National Marine Mammal Laboratory (NMFS)

(Seattle). One whale was tracked for 15 days. The fresh-water lens in the Bay refracted surface sounds downward and the strong currents of different water densities and temperatures in Icy Strait had reflecting interfaces between water masses, complicating the sonar tracking.

PLANS FOR FUTURE

Emphasis will be on the study of patterned sound sequences and on low frequency sounds and their correlations with whale movements and behaviors. We hope to be able to return for more detailed observations of sperm whales in the Southeast Caribbean. Sonar studies of whales underwater will be utilized at each opportunity and continued background observation of cetaceans in Cape Cod waters is planned. Radio tagging and tracking of three species in the local area is anticipated with BLM/MMS funding to try to characterize movements and distributions of these whales.

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BIOLOGICAL SIGNAL/NOISE PROGRAM

OPTICAL

BIOLUMINESCENCE MECHANISMS

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NR-

OBJECTIVES

To understand the following aspects of marine bioluminescence:

- (1) cellular processes regulating luminescence
- (2) membrane and neural regulation of luminescence
- (3) behavioral uses of bioluminescence by marine organisms.

ABSTRACTS

INSTRUMENTATION DEVELOPMENT: A fast spectrometer system has been developed and tested on shipboard. It consists of a PAR Optical Multichannel Analyser (OMA) and two quantum counters viewing the same space, allowing spectral measurements and determination of luminescence kinetics from bioluminescent sources. The OMA detector is an image intensified reticon and the spectrum is generated by a monochromator. Reliable spectra are recorded between 300 and 750 nm with resolution of the mercury 577/579 doublet. A single spectral scan requires 16 msc and these may be accumulated for up to 20 sec when very dim sources are under study. One quantum counter views the preparation through an interference filter of choice while the other measures a function of total light. Radiometric calibration is available while at sea.

In connection with the Sergestes counterillumination study, apparatus for electrophysiological investigation of the crustacean visual system has been developed and tested. Visual action spectra and light adaptation data are currently being measured via the electroretinogram.

BIOLUMINESCENT SPECTRA: Principal use of the spectrometer has thus far been during a cruise in southern California waters in which trawled material as well as collections made by Alvin were studied. Excellent spectra were obtained from about 40 species including many new to science. In September/October of this year we will take the system to sea again to study "gelatinous" midwater organisms collected with the WASP.

GNATHOPHAUSIA: Specimens of this organism lose the capability to luminesce after several months in the laboratory. Previously, we suggested that this is due to a non-specific dietary deficiency. Ms. Frank has now discovered that non-luminescent Gnathophausia do become luminescent again upon feeding of fresh trawled Triphoturus mexicanus, a myctophid. The luminescent spectrum of restored luminescence of T. mexicanus-fed Gnathophausia differs from that of T. mexicanus luminescence by being broader and extending more into the red while having the same λ max. Restoration of gnatho luminescence did not occur upon feeding with a variety of other possible luminescent sources.

SERGESTES COUNTERILLUMINATION: Current work involves determination of the action spectrum for initiation of counterillumination and light adaptation effects on this process. Ultrastructural work is in progress to determine if there is a circadian cycle of photoreceptor membrane renewal in Sergestes and if exposure to surface light levels causes damage to photoreceptor membranes.

DINOFLAGELLATE BIOLUMINESCENCE: An extensive study of the identity of luminescent microsources in Pyrocystis using image intensifier and fluorescence microscopy has been completed and published. We have now returned to the electrophysiology of luminescent excitation in this species and are correlating membrane electrical activity with the pattern of luminescence by means of simultaneous intracellular recording from specimens viewed with the image intensifier microscope. Variation in vacuolar pH is under study using pH sensitive dyes with the hope of obtaining further evidence regarding the proton theory of excitation of dinoflagellate luminescence.

A new approach to the natural mode of luminescence excitation is being made by observing the detailed pattern of bioluminescence around spheres moving through a uniform field of Gonyaulax at velocities around that inducing turbulence. With the image intensifier-video system, instantaneous velocity can be measured, and each dinoflagellate flash can be determined over half of the sphere and in its wake.

CHAETOPTERUS: In conjunction with a study of the interaction of chemical and particulate stimuli in feeding by this tubiculous annelid, the hitherto obscure role of its secreted luminescence has been examined. Photomultiplier and image intensifier recording of luminescence during various types of natural stimulations have been made. These show that a luminous "cloud" is formed in the tube prior to ejection of water contained in the tube. Ejection is caused by various noxious stimuli and is accomplished by a rapid posterior to anterior contraction of the worm with fans erect.

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DINOFLAGELLATE SWIMMING BEHAVIOR IN RESPONSE TO CHANGES
IN TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS

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OBJECTIVES

(a) To determine dinoflagellate swimming behavior in response to changes in temperature, light and chemical factors, (b) to use a versatile mathematical formulation to statistically describe the swimming characteristics of different dinoflagellate species, (c) to derive regression relations of how swimming speed varies with size within the dinoflagellates.

ABSTRACT

Field studies of dinoflagellate diurnal vertical migration are based on population measurement techniques that ignore the behavior of individual cells within the species population. Though the population signal may represent the successful mean behavior of the species at a given time, individual cells within the population apparently exhibit a range of swimming speeds and of environmental responses that are influenced by cell state and, possibly, the genetic variability within the population. Further understanding of the ecological role of dinoflagellate motility requires statistical information on how swimming speed and orientation vary within a species population among varying environmental conditions.

Video camera microscopy will be used to measure the velocity of selected dinoflagellate species representing the full size spectrum of the group under a variety of temperature, light and chemical conditions. These various measurements will better describe the motility character of individual dinoflagellate species, the motility differences among species and the time course of dinoflagellate motility under a simulation of encountered environmental variability.

The analysis of these motility measurements is accomplished through the use of a new empirical expression

$$R = R_A (1 - e^{-a(P - P_L)}) (1 - e^{-b(P_H - P)})$$

where the various terms are R-rate process, R_A -asymptotic rate process, a-initial slope, P-property value, P_L -lowest property value, b-final slope, and P_H -highest property value. One application of this expression describes a curve that fits the whole range of temperature versus swimming speed capabilities of a given species. Statistical fits of this expression to swimming speed data for various species

provides a robust empirical measure of how dinoflagellate swimming speed varies within this group of microalgae.

The results of this study will provide a new perspective that can be used to predict dinoflagellate distribution, density and species characteristics. To date the physiology of dinoflagellates has been emphasized. In nature, environmental variability combines with organism behavior to set the exposure regime that elicits the physiological response. Behavior, even in phytoplankton, is a third modifier of the natural response.

PLANS FOR FUTURE

(a) To continue development of a video camera microscopy system that measures the velocity of diverse dinoflagellate species, and (b) to further examine the motility relationships within the dinoflagellate class of microalgae.

BIOLUMINESCENCE: MEASUREMENTS AND ORGANISMS
IN THE UPPER 600M

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OBJECTIVE

(a) To compare bioluminescence measurements obtained using two types of bathyphotometers: An open system in which the photomultiplier tube (PMT) in its pressure housing was mounted to accept all light (bioluminescence and sky shine) approximately 45 deg incident to the viewing window. Secondly, a pumped system which measured bioluminescence of turbulently flowing seawater from a light baffled chamber.

(b) To measure and correlate the transition of bioluminescence and organisms from a surface water (upper 100M) environment to midwater (below 200M) environment.

ABSTRACT

Two bathyphotometers were mounted on the forward frame of the submersible Johnson Sea Link II (JSL II) with the readout and detector electronics mounted in the aft diving compartment. The PMR's (RCA 8575) were operated in photon counting mode. The data readout consisted of consecutive PMT counts/50 sec time bins (giving average intensities) and high time resolution scans of bioluminescence event kinetics (counts/10 msec time bins).

Two dives to a depth of 600M were made on the moonless night of June 21-22, 1982, at a location approximately along the western edge of the Gulf Stream (27 deg 27.6'N, 79 deg 34.6'W). Data were obtained with both detectors and organisms were collected. Surface light penetration was observed on the open detector to a depth of 200M; above 150M the light obscured the bioluminescence. Bioluminescence measured with the pumped detector showed most activity above 120M. Below 200M, both detectors showed relatively little activity overall with a small layer at about 330-360M. The detector measurements were comparable below 200M with the open detector showing more signal. Bioluminescence events at depth were generally longer in time than those in surface waters.

Examination of collected plankton samples revealed the presence of the dinoflagellate genera Ceratium, Ceratocorys, Peridinium, Ornithocercus, Podolampas, and Pryocystis in the upper 40M. Below 40M, the number of dinoflagellates collected dropped markedly. Calanoid, cyclopoid, and harpacticoid copepod densities were greatest in the shallowest sampled layer (20M) and decreased by one order of magnitude at 150M. In general, the total number of collected copepods (with a 35 micron mesh netting) mirrored the vertical bioluminescence intensity profile (pumped bathyphotometer). Plankton samples collected from the migrating layers (275M and 400M) appeared to consist of the small cyclopoid

copepods Corycaeus, Oncaea, and Oithona. Numerous nectophores of the siphonophore Vogtia serrata, which we believe to be luminescent, were collected at 400M. At a depth of 400M, light from a flash light was pulsed into the seawater from the forward sphere. Luminescent organisms responded with a large luminous display. This effect diminished with shallower depths and was nonexistent above 60M.

PLANS FOR FUTURE

Additional dives using the JSL II as well as other submersibles are planned. ALVIN will be used in November, 1982, and July, 1983, to measure bioluminescence to greater depths and to sample organisms. JSL II will be used in August, 1982, to obtain similar measurements in addition to sample and measure "marine snow". We also plan to investigate light flash stimulus response of mesopelagic and bathypelagic organisms from manned submersibles.

CHLOROPHYLL a VARIABILITY IN OCEANIC MIXED LAYERS

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N00014-80-C0098

OBJECTIVES

(a) To exploit the use of chlorophyll a as a tracer of processes and dynamics of oceanic mixed layers, (b) to study the relationship between physical processes and phytoplankton growth in the sea.

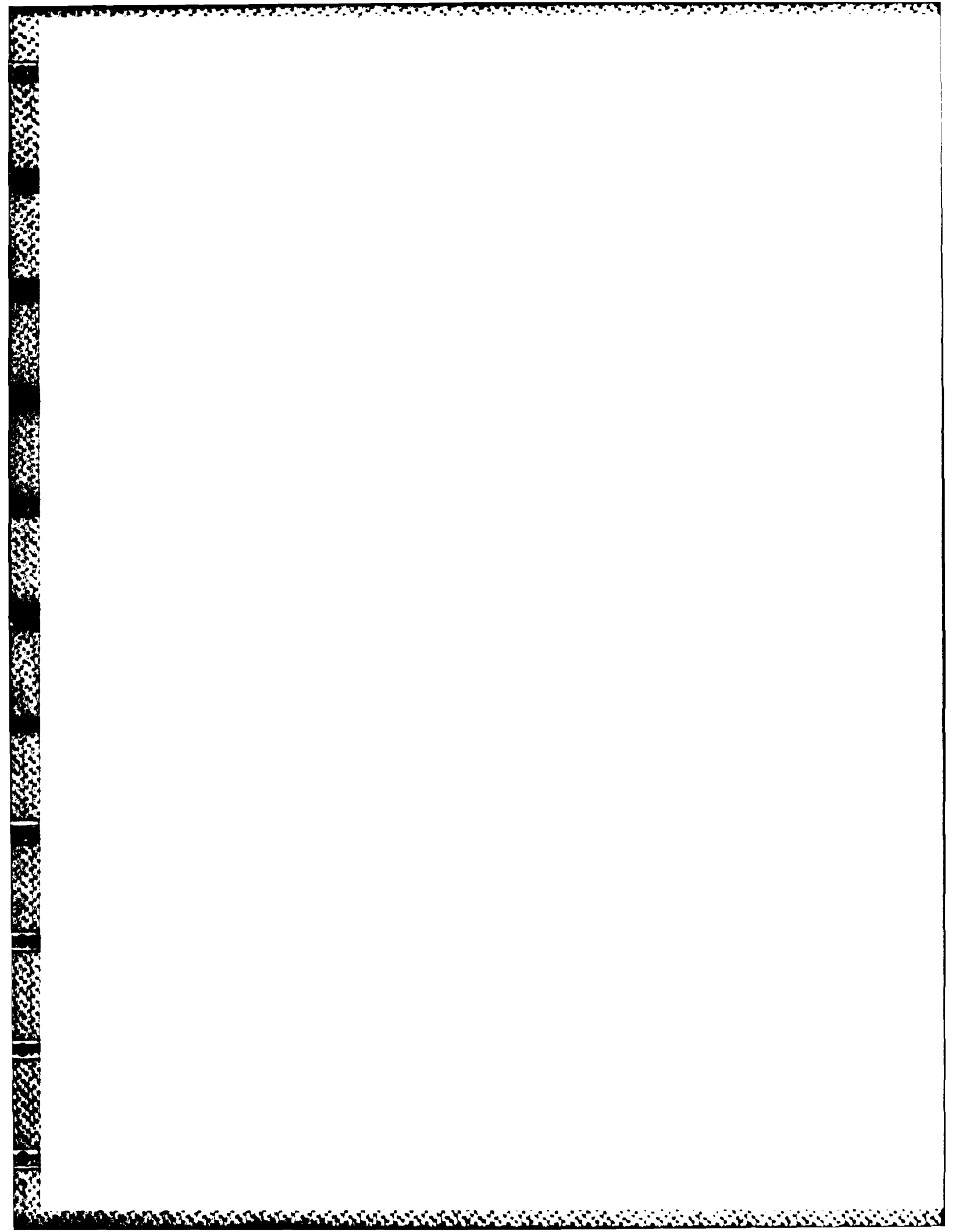
ABSTRACT

The approach used in this research is based on the idea that if it is possible to understand the mechanisms controlling the availability of substrates (nutrients and light) for phytoplankton growth, we will be better able to predict distributions, production and population composition of phytoplankton growth in the sea. Also, we will provide information which can be used by physical oceanographers for a hydrographic situation that provides few clues to dynamics. The working hypothesis for this research is that chlorophyll a is a passive contaminant in stirring and mixing motions in the mixed layer and pycnocline on time scales important to mixed layer dynamics.

As of this writing we have assembled an in situ fluorometer package (fluorometer, data logger, battery pack) to be suspended beneath a current meter designed to measure vertical currents (R. Weller, J. Price: WHOI). The fluorometer package will also measure and log water temperature and pressure. Calibrations and testing are now in progress.

PLANS FOR FUTURE

A shakedown cruise aboard R/P FLIP for equipment testing is scheduled for December, 1982. The full experiment, with FLIP and R/V WECOMA is scheduled for Oct-Nov., 1983. During 1983, (a) we will evaluate the in situ fluorometer package and make design changes where necessary and (b) assemble a system for mapping the variability in surface chlorophyll and sea surface temperature, to be used aboard WECOMA during the Oct-Nov., 1983 experiment. With these additional measurements, we hope to characterize the large scale variability in hydrography and chlorophyll a with which to interpret data collected aboard FLIP.



CENTRAL GYRE RESEARCH PROGRAM

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NR 083-005

OBJECTIVES

Our objectives are to understand the physical and biological driving forces that are responsible for regulating the community structure of a pelagic ecosystem. We are asking the questions: of all of the possible configurations, why does the central gyre have the observed one and what forces will change this structure?

ABSTRACT

In order to understand the dynamic forces that regulate both species and trophic structure of any community-ecosystem it is necessary to have an adequate, quantitative description of the structure and its stability in time and space. We feel that we now have such a description for the biotic half of the system and have made significant progress in describing the relevant aspects of the physical half.

The biological structure is extraordinarily stable. We have compared measurements made at sample separations of 100s of meters to 1000 km and 0.5 hrs to decades and find no significant changes in several different indices of community structure. The same kinds of measurements made in the California Current show great changes at sample separations of 10s of km and hours. Thus the gyre community is highly coherent in space and time. It is also a resilient system. There have been widespread and significant physical perturbations during the time we have been studying the gyre and yet the species dominance structure remained the same. This strongly implies that the forces regulating dominance are strong and are biological in nature. However, we have tested two important theories that "explain" species dominance structure. These are "resource allocation" and "perturbation-disturbance" theory. Neither of them provide adequate explanations for our observations.

Since the carrying capacity of the gyre depends on the rate of nutrient input, we are examining this process as well as structure. Again we find that standard "explanations" (winter mixing; horizontal advection) are not adequate. We are analyzing a large amount of nutrient and hydrographic data in order to obtain a more detailed picture of nutrient and physical structure in time and space.

PLANS FOR THE FUTURE

We have made substantial progress in the study of structure and function in our ecosystem. The data, now in hand, are sufficient for tests of three important ecosystem theories. Our plans for the near future are to write up and publish our biological results and to continue the analyses of the nutrient-physical data. Eventually we expect our results to lead to plans for further experiments at sea.

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- Fiedler, Paul C. Zooplankton avoidance and reduced grazing responses to Gymnodinium splendens (Dinophyceae). Limnol. Oceanogr., In Press.
- Hayward, T. L. 1981. Mating and the depth distribution of an oceanic copepod. Limnol. Oceanogr., 26, pp 374-377.
- Hayward, T. L., and John A. McGowan. 1981. The shallow salinity minimum and variance maximum in the central North Pacific. Deep-Sea Res., 28(10), pp 1131-1146.
- Hayward, T. L., and E. L. Venrick. The relation between surface chlorophyll, integrated chlorophyll and integrated primary production. Mar. Biol., In Press.
- Hayward, T. L., E. L. Venrick, and J. A. McGowan. Environmental heterogeneity and plankton community structure in the central North Pacific. Submitted to J. Mar. Res.
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- Venrick, E. L. 1982. Phytoplankton in an oligotrophic ocean: Observations and questions. Ecol. Monogr., 52(2), pp 129-154.

MECHANISMS OF BIOLUMINESCENCE: KINETIC-SPECTRAL AND BIOCHEMICAL
PROPERTIES AS A PROBE OF MARINE COMMUNITIES AND INTERACTIONS

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OBJECTIVES

(a) To develop and deploy a prototype offshore station for continuous monitoring of bioluminescence activities, (b) to use this station to make continuous temporal measurements of undisturbed (background) luminescence on both short and long time scales, (c) to develop algorithms and computer software capable of distinguishing different luminous flashes and processing the bioluminescence data, (d) to characterize the luminescence of a variety of organisms with regard to spectral and kinetic properties, developing three-dimensional luminescent signatures for each organism or group of organisms, (e) to study physiological and biochemical properties of a variety of luminous organisms, correlating these activities with those expected in oceanic environments.

ABSTRACT

A station for continuous monitoring of bioluminescence has been fabricated and deployed in Scripps Canyon, approximately 1 mile offshore from Scripps Institution of Oceanography. The sensory unit, which consists of phototubes looking upward and downward, can be placed from subsurface to 200 m depth. It is connected to the laboratory via a hard wire link that provides electrical power and telemeters the collected data at high rates back to the laboratory. In the lab data are primarily stored in digital form on the mainframe computer that is also capable of analyses of several of the parameters of luminescence. With the photometer array at 100 m, we have recorded luminescence activities on a regular basis during the last 10 mo. These data have been analyzed both in strip chart format by hand and in digital form by computer. Agreement between these methods is good, indicating that the software we developed to process the digital records is reliable and useful for interpretation of the bioluminescence. Current data analyses have so far addressed the total number of luminous flashes per unit time and the separation of distinct classes of flash types by the duration of the flash. Using these methods we have seen some striking features in terms of the pattern and variation and levels of bioluminescent activity throughout the night, month and season. Total flash rates generally increase during the early hours of the night and then decrease rapidly through the middle of the night, increasing to high levels in the early hours of the morning. Different levels of bioluminescent activity were seen above and below the plane of the photometer array, but the reasons for these differences are not yet elucidated.

The data set has also been analyzed for specific flash types determined by the duration of the bioluminescent event. Early studies showed distinct temporal variations in flash type and suggested the possibility that our methods can be used to study differential activities of the organisms responsible for each temporal flash type. In concert with these analyses, midwater trawls were made in the vicinity of the station during periods of luminescent activity in an attempt to identify the organism(s) responsible for each type of flash. The predominant species were dinoflagellates, although some larger luminescent organisms (euphausiids and sergestids) were also netted. These organisms were transported to the laboratory and the kinetics of their bioluminescent flashes recorded. The predominating signal in the field data had temporal features matching those of the dinoflagellates, while the longer records matched those of the euphausiids.

In the laboratory we have documented the kinetic-spectral features of bioluminescent activity of these and other organisms. When intensity, color and time of a luminescent event are recorded and displayed in three dimensions, recognizable signatures of various organisms can be seen. Such signatures have been collected from 14 species of marine organisms and represent the first compilation of luminescence temporal-spectral signatures from a variety of organisms. The device used to study these events, a polychrometer, was designed and built in our laboratory and is a prototype of submarine and open ocean models. The polychrometer has also been used to study luminescence in cell-free systems, analyzing color and kinetics of enzymatic light emission under a variety of different conditions.

We have elucidated, at the molecular level, the mechanism by which bacteria control their own luminescent activities. A small molecule (N-(β -ketocaproyl) homoserine lactone) was purified, identified and synthesized. This molecule, which is produced by the luminous bacteria, was shown to be responsible for the level of light emission in these organisms and was hypothesized to account for fluctuations in levels of bacterial luminescence in various oceanic environments. Physiological studies using chemostats have verified this model and predicted that planktonic luminous bacteria should be dark, while particle-associated and symbiotic forms should be luminescent.

We have purified and characterized a protein responsible for the emission of yellow light by some luminous bacteria. This 22,000 molecular weight protein, called YFP (yellow fluorescent protein), when added to extracts of the light-emitting enzyme, luciferase, causes a 50-nm red shift in luminescence, resulting in a yellow light emission. The color of the light is sensitive to aldehydes, temperature, pH and salt concentration in vitro.

We have studied several symbiotically luminescent systems in which bacteria are used by fish or squids to supply light for their hosts. We have shown that these symbioses act as continuous sources of inoculum of luminous bacteria into the ocean and that these bacteria are initially luminous, but lose their luminescence after several hours of dilution into sea water. Such symbioses may thus contribute to the actual and potential luminescence of oceanic environments and may be indicators for populations of symbiotically luminous fish or squid.

We have cloned the genes for bioluminescence (luciferase genes) in order to understand, at the molecular level, the mechanisms of control and activity of bioluminescence. In this way we have verified the presence of the bacterial luciferase genes in the bacteroids of some luminous fishes, even though it was not possible to culture the bacteroids and study them independently.

PLANS FOR FUTURE

(a) To add a winch to the field station to allow measurements at any specified depths, (b) to develop software for kinetic-spectral signature analyses, (c) to alter the photometer array for distinguishing background from stimutable bioluminescence, (d) to use the modified field station to study the vertical migration of identifiable luminous species in Scripps Canyon, (d) to perform laboratory studies of biochemical mechanisms of light emission, and physiological control over luminous activity

CELL DIVISION FREQUENCY AS A MEANS OF ASSESSING
NATURAL POPULATION GROWTH IN MARINE PHYTOPLANKTON

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NR 083-530

OBJECTIVES

The goal of this research is to evaluate cell division frequency (CDF) as a means of determining in situ growth of natural populations of marine phytoplankton. Special emphasis is placed on: (a) establishing cell division frequencies for natural phytoplankton populations in coastal Gulf of Mexico and Beaufort, N.C. waters; (b) evaluating various culture methods and choosing appropriate algal species for comparing the CDF method with conventional cell count techniques; and (c) testing the CDF method relative to selected environmental variables in culture. The results of our study should extend our ability to access instantaneous growth activity in natural populations of phytoplankton by a basic feature - mitotic index serving as the integrator of environmental conditions impinging upon the sampled populations.

ABSTRACT

The first year results showed that the field collected marine diatoms, Coscinodiscus radiatus, Guinardia flaccida, Rhizosolenia alata and R. styliformis were able to provide adequate nuclear division figures for cell division frequency or mitotic index calculation. The mitotic index information provided statistically significant growth rate data. Considerable replication of slide counts and daily sampling served as the data base for statistical treatment. The percent of the population dividing, specific growth rate and doubling time was generated from the mitotic index data for each diatom species studied.

The second year, we made a study of accuracy within the mitotic index technique under controlled laboratory conditions and compared the mitotic index technique with that of the traditional cell count method. Replicate cultures of the marine diatom Biddulphia mobiliensis exhibited extremely similar mitotic index values between slide counts and flasks under similar growth conditions. A consistently large variation for the cell count method was noted with several replicate slide counts.

To meet our third year objective we have tested for possible sensitiveness and reliability of the mitotic index method in the early detecting of environmental stress in cultures of near natural populations of marine diatoms at the Duke Marine Laboratory (DURL), Beaufort, N.C. The study was designed to analyze the effects of various copper concentrations on cell division frequency of a selected marine diatom, Biddulphia sp., in a mixed culture of phytoplankton and zooplankton, as found in the Beaufort Channel at that time. A series of baseline studies was carried out to quantify mitotic index values at varying intervals during a 24-hour period for multiple growth flasks under identical culture conditions. The results of these tests will establish the variability to be found within replicate cultures.

The mechanism of hormesis within the test alga was also investigated with the help and guidance of Dr. Brenda Saunders, DURL. Hormesis is the name given to the stimulatory effects caused by low level of potentially toxic agents as in this case, copper.

Laboratory data collected between May and July, 1982, are presently being processed and analyzed in the attempt to demonstrate subtle changes in cell division frequency by the mitotic index method in stressed populations of marine diatoms.

PLANS FOR THE FUTURE

In the remainder of our final year we plan to study additional marine diatom species in mixed culture under varying culture conditions such as temperature fluctuations, light intensities, photoperiod and nutrient manipulation in attempts to further quantify early instantaneous growth rate changes using the mitotic index method.

CURRENT REPORTS AND PUBLICATIONS

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PATCHINESS IN OCEANIC BIOLOGY AND PHYSICS

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104-246

OBJECTIVES

We are attempting to produce very detailed descriptions of the abundances and spatial distributions of organisms on fine spatial scales (order 10s of cm), to correlate those distributions with physical oceanographic finestructure, and to use the biodistributions to study aspects of turbulent processes in the upper ocean which the physical oceanographic community is unable as yet to measure directly.

ABSTRACT

We have now developed and sea-tested our complete towed video/CTD system which simultaneously measures fine-scale distributions of organisms (categorized as to size-frequencies), T, S, chlorophyll-a fluorescence, volume reverberation at 3 MHz, and other environmental parameters. Organisms are recorded by a video camera, using strobed lighting at right angles to the camera's line of sight. Video pictures and all environmental data are recorded on a video tape recorder as well as displayed in real time on a monitoring deck unit. The deck unit also serves as a playback system for recorded tows. The system includes its own winch and cable and can be deployed from any ship having 220/440 vAC and an A-frame or heavy davit. It can operate at several knots for over 10 hours on one battery charging, and may be towed horizontally, "tow-yo"ed, or used vertically for profiling.

We went to sea in April on an engineering shakedown cruise. The system worked well, and we had extremely clear weather (due to a "Santa Ana" condition, a "foehn" wind). We obtained extensive IR and color satellite coverage of our study area (near Catalina Is. off S. Cal.) through the SIO Satellite Facility, and used those data (plus a \$5 bucket thermometer) to locate a pronounced thermal/color front. We made numerous tow-yo hauls and horizontal hauls on either side (and through) the front; we obtained about 450,000 video images plus 20M bytes of digital data. We have been busy figuring out how to handle such massive data sets, and are working on the image analysis problem.

We observed no development of a surface isothermal layer despite a 12+ hour 30+ kt wind event; we also saw no evidence of nighttime convective overturn despite optimal conditions. We did find pronounced chlorophyll and zooplankton concentration changes on very short spatial scales (order a few 10s of m); those changes do not appear correlated with observed physical mixing or advective processes. We have data suggesting that on local scales chlorophyll may well behave as a reasonably conservative property; we have parallel data which indicate that local grazing effects upon chlorophyll may be quite severe. We are further investigating both possibilities (ms in prep).

FUTURE PLANS

We are working on the image analysis problem. The image analysis community/industry is only now beginning to address questions such as ours, to wit, the very rapid (simple-minded) analysis of very large numbers of clear, simple pictures. We

intend to go to sea again soon, and to use the system to (a) describe distributions of organisms on very fine scales; (b) to use the physical measurements to infer causes for those distributions; and (c) to use the distributions of organisms to study the development and decay of near-surface turbulent events such as Langmuir cells and convective overturn.

CURRENT PUBLICATIONS

Shulenberger, E and JL Reid (1982) The Pacific shallow oxygen maximum, deep chlorophyll maximum, and primary productivity, reconsidered. Deep-Sea Res. 28A:910-919.

Shulenberger, E (1982) Biological evidence for an E-W split in the North Pacific Central Gyre. Deep-Sea Res. 29A:403-410.

Greenblatt, PR, E. Shulenberger and JH Wormuth (1982) Small-scale distributions of zooplankton in the California Current. Deep-Sea Res. 29A:443-457.

Haury, LR and E Shulenberger (1982: accepted) Horizontal transport of phosphate in the California Current. California Co-Operative Oceanic Fisheries Investigations REPORTS: 23.

Shulenberger, E (submitted) Effects of melting icebergs on water-column biology nearby.

Shulenberger, E and RE Lange (submitted) Mixed layer vs mixing layer and Sverdrup's concept of critical depth.

Lange RE, E Shulenberger and BP Johnson (in prep) A nearshore thermal front investigated with a new towed physical/biological sensing system.

ESTABLISHMENT OF A FLOW CYTOMETRY AND CELL SORTING CENTER

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NR 083-058

OBJECTIVES

To develop a national resource laboratory for ocean biology with regard to flow cytometry and to develop and validate protocols applicable to ocean organisms.

ABSTRACT

There are numerous reasons for probing cells that retain full integrity, metabolic capacity and viability. Cytofluorometry is an automated method of assessing the biomass and frequency of cell division in nano- and picoplankton and single cells isolated from multicellular organisms. The fluorescence spectral signatures of pigmented, colorless and dividing cells can be determined using autofluorescence and appropriate fluorescent stains.

Biochemical and biophysical cellular properties of individual cells as determined by direct quantitative measurements are becoming increasingly important. Vast improvements in the optics, electronics, stain utilization and immunofluorescence result in the ability to analyze quantitatively large numbers of cells (e.g. 10^3 to 10^6) of a population within minutes. Advancements, impossible by other means of analysis include: quantitation of multiple parameters; analysis on individual cell basis; analysis at the rate of over one-thousand cells per second; good sensitivity; statistical precision and cell sorting capability enabling the researcher to conduct other analyses of the flow cytometrically separated cells.

Flow cytometry and cell sorting has received widespread acceptance in biomedical research where measurements are now routine. With sufficient adaptations of protocols to marine microorganisms and single cells from multicellular organisms, this methodology is expected to be highly useful for marine biological research. By concentrating efforts in one physical location, an expertise can be developed over time which will permit the training of scientists from other laboratories. We stress that it takes a considerable amount of time to develop the expertise in flow cytometry, and by having the Bigelow Laboratory as a focal point for this development

it would facilitate the application of this technology in other locations. Thus, Bigelow Laboratory would become a national resource laboratory for ocean biology with regard to flow cytometry.

At Bigelow Laboratory, there will be a formal designated research room with equipment dedicated to flow cytometry so that visiting investigators can feel free to carry on their investigations without imposing on other ongoing research programs and space. Users of the FLOW CYTOMETRY AND CELL SORTING CENTER will have easy access to the NSF-funded Culture Collection of Marine Phytoplankton as well as natural populations from temperate coastal waters of the Gulf of Maine.

PLANS FOR FUTURE

We will develop a Flow Cytometry and Cell Sorting Center at the Bigelow Laboratory for Ocean Sciences in order to a) develop and validate methods applicable to ocean organisms, and b) train scientists from other laboratories.

CURRENT REPORTS AND PUBLICATIONS

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BIOLOGICAL SIGNAL/NOISE PROGRAM

CHEMICAL

MICROBIOLOGY OF DISSOLVED GASES

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N00014-79-C-0004

NR 083-102

OBJECTIVES

(a) to identify the specific microorganisms involved in the production and consumption of H_2 in oceanic waters, (b) to identify the specific chemical and physical parameters affecting production and consumption of H_2 , (c) to identify the biochemical linkages between H_2 and the production and consumption of other trace gases such as CH_4 , CO and N_2O , trace metals such as manganese and iron, and other compounds such as ammonia and hydroxylamine, and (d) to measure the in situ rates of production and consumption of H_2 , CH_4 and CO.

ABSTRACT

Initially, we believed that N_2 -fixing microorganisms, and particularly cyanobacteria were the principal source of H_2 in oceanic waters. Although N_2 -fixation was detected in estuarine waters and found to be associated with H_2 production, we have not been able to demonstrate that N_2 -fixation is a significant source of H_2 in open ocean waters. Other known microbial species that produce H_2 , such as sulfate reducers, anaerobic heterotrophs, and some methanogens, were also not detected. Consequently, we formulated a set of hypotheses in which H_2 could be microbially produced by incomplete oxidation of CH_4 , NH_4^+ , NH_3 and possibly reduced organic compounds. In oceanic and estuarine environments where peaks of H_2 are found, peaks of CH_4 and CO are frequently found as well. The most pronounced zones where these gases have been measured are associated with oxyclines such as found in anoxic fjords, or in the oxygen minimum zones in the Tropical Pacific or off the coast of Oregon. This would imply that the levels of dissolved O_2 in the water determines the extent of oxidation of these compounds. Both H_2 and CO could result from the incomplete oxidation of CH_4 , for example. Similarly, H_2 and hydroxylamine could result from incomplete oxidation of ammonium.

Pure cultures of CH_4 and NH_4^+ oxidizing bacteria have been isolated from waters obtained during cruises to the Tropical North Pacific, off the coast of Oregon and to Saanich Inlet, British Columbia. These isolates have been cultured in chemostats under conditions where levels of the energy sources and O_2 can be regulated. These preliminary results confirm that when the O_2 is reduced to the minimum allowable for growth, that CO and H_2 are produced from CH_4 , and H_2 is produced from NH_4^+ . These experiments are being confirmed with other isolates including species that can simultaneously oxidize more than one inorganic energy source and reduced gases and grow with generation time of 4 to 5 hr at 20 C, and some similar isolates from submarine hydrothermal environments.

Thermophilic bacterial communities have been cultured from the super-heated waters associated with submarine hydrothermal environments. These communities were found to produce H_2 , CH_4 and CO , and consume CH_4 at 100 C at one atm. We have also demonstrated that these thermophilic communities can grow chemoautotrophically at temperatures from 150 to 300 C at 265 atm in a high-temperature/high-pressure growth chamber.

These preliminary findings indicate that the bacteria from submarine hydrothermal environments which are involved in the production and consumption of gases are physiologically analogous to non-vent open ocean bacteria which carry out similar biochemical processes. The important parallel finding is that the aerobic vent bacteria are growing under conditions in which the O_2 and other electron acceptors such as NO_3^- are either not present or are limiting.

PLANS FOR FUTURE

(a) Test the hypothesis that an important source of H_2 in open ocean waters is the result of incomplete oxidation of CH_4 , NH_4^+ and possibly organic compounds.

(b) Construct and field test a water sampler for measuring in situ rates of production and consumption of trace gases.

CURRENT REPORTS AND PUBLICATIONS

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MICROBIAL PRODUCTION OF NONCONSERVATIVE GASES IN OCEANIC SURFACE WATERS

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N00014-80-C-0113

OBJECTIVES

(a) To determine the association of H_2 and N_2O producing bacteria with shallow oceanic nepheloid layers, (b) to isolate and identify the producing microorganisms, (c) to measure the rates of gas production under varying conditions, (d) to enumerate the in situ number of microorganisms producing each gas.

ABSTRACT

We have previously measured H_2 and N_2O production by shallow, nepheloid layer-associated bacteria obtained from eastern tropical North Pacific, Caribbean Sea, Mediterranean Sea, and Gulf of Mexico waters. The nepheloid layer water samples were concentrated using a reverse-flow filter (3 μ m Nuclepore) developed for the project. Concentrated water samples were incubated anaerobically in the dark and headspace gases were periodically analyzed by gas chromatography. Results indicate that N_2O production proceeds through a denitrification process and N_2O can be released into the water column when in situ nitrate concentrations are adequate. H_2 production in our system appears to occur through a fermentative process and is stimulated by nutrient supplementation. The organisms responsible for production of both gases appear to be ubiquitous within the temperate and tropical waters sampled.

Current research involves the isolation and identification of the microorganisms responsible for the observed gas production and measurement of the rates of production for pure and mixed cultures under simulated in situ conditions. We are also developing the methodology for identifying and enumerating specific types of bacteria, . e., immunofluorescence. Antibodies are being produced against specific H_2 and N_2O producing organisms for subsequent use in identifying and enumerating specific types in natural water samples.

We have also measured H_2 and N_2O production in the Sargasso Sea during a June 1982 cruise aboard the USNS LYNCH. These gases were detected in both concentrated and unconcentrated water samples. Very high concentrations of H_2 were measured in the headspace gases (ca. 6% V/V) and N_2O production was minimal in the absence of added nitrate.

PLANS FOR FUTURE

(a) To isolate and identify all organisms collected, (b) to produce antibodies against all pure cultures, (c) to enumerate specific bacterial types through immunofluorescence techniques, (d) to estimate the in situ H_2 and N_2O production rates under given conditions.

CURRENT REPORTS AND PUBLICATIONS

- S. J. Schropp and J. R. Schwarz. 1982. Hydrogen production in the Mediterranean Sea. *Proc. Amer. Soc. Microbiology*
- S. J. Schropp and J. R. Schwarz. 1982. Nitrous oxide production by denitrifying microorganisms from the eastern tropical North Pacific and the Caribbean Sea. *Geomicrobiol. J.* In press.
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BIOTURBATION

MICROBIAL ACTIVITIES IN THE DEEP SEA BENTHIC BOUNDARY LAYER

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OBJECTIVES

Microbial transformations in offshore top sediments are measured in situ with the aim to assess a) decomposition rates of organic substrates (fatty acids, hydrocarbons), b) nutrient regeneration, c) microbially mediated corrosion (sulfate reduction, sulfide oxidation), d) pressure and temperature effects on microbial activities in general, and e) interactions between epibenthic invertebrates and their intestinal microflora. Fully automated free-vehicles are used for the injection and recovery of radiolabeled substrates in the top deep sea sediment layers for in situ incubation and recovery for shipboard and laboratory analyses.

ABSTRACT

A number of important bio-geochemical reactions as well as the life of benthic and epibenthic animals on the deep sea floor in general are dependent on the occurrence and rates of various microbial transformations. These activities start with the oxidative decomposition of sedimenting organic materials and lead via oxygen depletion to the anaerobic respiration of nitrate, sulfate and carbon dioxide and, in some cases, specific fermentative decomposition of organic compounds, to the final re-oxidation of the reduced C, N and S compounds (such as CH_4 , NH_3 and H_2S) in the oxygenated benthic boundary layer. Well known from shallow water studies, the occurrence and rates of those transformations in deep sea sediments are practically unstudied. Our five-year effort in this area led to the successful development of a relatively inexpensive, timer-operated, free-vehicle system for the in situ measurement of microbial activities. It has been a major objective of this research to extend the measurements over a number of geographically, geologically and biologically different types of sediment. To this end, sediments rich in organic compounds (Bermuda Rise) and those of low concentrations of organic carbon (Bermuda Basin) as well as sediments near the Northwest Atlantic continental slope (DOS I and DOS II) have been studied and the data compared to shallow water deployments (Buzzards Bay). During fiscal year 1981/82 data on the microbial utilization and carbon incorporation from glucose, mannitol, lactate, pyruvate and trimethylamine have been collected from four more tripod deployments. In all cases the data from the in situ incubations are also compared to control measurements done in parallel sediment samples retrieved and taken into the laboratory for incubation at 1 atm and controlled temperature. In addition, data on the uptake of radiolabeled protein by the intestinal microflora of deep sea amphipods as compared to the portion taken up by the animal tissue have been gathered from two more tripod deployments. In

preparation of these experiments, we produced our own radiolabeled bacterial protein by extraction from cells grown on commercially available C¹⁴-labeled glutamic acid. Since FY 1980 we also conducted a study on deep sea bio-fouling. In parallel to tripod deployments bio-fouling racks have been deposited by DSRV ALVIN for a two years' exposure at the St. Croix Station (3990 m). The data on the microbial sulfate reduction are the first ones obtained from deep sea sediments and support Jorgensen's predictions.

PLANS FOR FUTURE

Consistent with our major research objective to extend our work over a larger range of sediment types, we have planned a cruise to the Newfoundland and Labrador Basins during the late summer of fiscal year 1982. According to available oceanographic and geological data, the sediments of those basins are strongly affected by the Labrador Current and substantially different from those of the Atlantic abyssal plain. With a repeat of this cruise in September 1983, the field work of this project will be concluded. The remaining time reaching into 1984 will be devoted to calculating and publishing our data.

CURRENT REPORTS AND PUBLICATIONS

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- Harwood, C. S., H. W. Jannasch and E. Canale-Parola. 1982. "An anaerobic spirochaete from deep sea hydrothermal vents". Appl. Environ. Microbiol. 44, 234-237
- Jannasch, H. W., C. O. Wirsén and C. D. Taylor. 1982. "Deep sea bacteria: Isolation in the absence of decompression". Science 216, 1315-1317.
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BIOLOGICAL CHARACTERIZATION OF THE
HEBBLE SITE: THE FAUNA

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083-231

OBJECTIVES

(a) To characterize the spatial variability of the biota of the HEBBLE site, and (b) to translate that description into measures of the impact of the biota on the parameters of a sediment transport model.

ABSTRACT

The High Energy Benthic Boundary Layer Experiment (HEBBLE) is an investigation into the dynamics of the benthic boundary layer in an area where near-bottom current velocities are high. HEBBLE's goal is to develop a model of sediment transport in the deep ocean. Sediment transport parameters include critical erosion velocity and hydrodynamic roughness length. Organisms alter the sediment fabric and surface in ways that affect these parameters. For example, deposit feeders ingest silt- and clay-sized particles, remove a portion of their microbial and organic coatings, and egest them as sand-sized fecal pellets or casts frequently on the sediment surface. Fecal casts, animal tracks, tests, and tubes create microtopography. Burrowing animals appear to decrease compaction and increase the water content of the sediment. Animals add mucus to sediments in their feeding and locomotion; bacteria use mucus to bind to sediment particles. All of these processes are dynamic in that the effect on the sediment of any one organism is being reversed by others. Clearly, organisms alter the tendency of particles to stick to each other, their packaging (i.e., bulk density), the hydrodynamic roughness of the surface, and the compaction of the bed in ways that are relevant to sediment transport. As a result, HEBBLE has a two-phase biological program (1) the characterization of the biota of HEBBLE site and (2) the use of shallow-water analogs in laboratory flume experiments to assess the importance of the biota to HEBBLE parameters.

To design the sampling program for phase 1, we required preliminary estimates of variability. We took two 0.25-m² box core samples in the general HEBBLE area in May, 1980, that were partitioned in situ into 25 100-cm² subcores nine of which from each core were used for

biology. All subcores, in turn, contained a 23-cm² subsubcore for bacteria and particle samples. From the 18 77-cm² X 10-cm deep samples, I have measured biogenous surface structures and sorted out the animals to provide initial estimates of functional group standing stocks and their spatial variability.

The results show that although the animal standing stocks are low compared to those found in shallow water, they are an order of magnitude higher than comparable samples from the deep North Pacific. The harpacticoid copepods are particularly interesting. In deep-sea localities, harpacticoids have a broad range of life styles and associated morphologies. However, the HEBBLE harpacticoid fauna appears to lack surface dwelling species. Further, many species have conspicuous adaptations for burrowing. The high near-bottom currents in this area provide a possible explanation for these observations.

The results from the preliminary samples were used to design the July, 1982, biology sampling program. Sixteen navigated box cores of high quality were taken in a stratified random design to be used for the characterization of the fauna of the HEBBLE site and its spatial variability.

FUTURE PLANS

I plan to (a) finish the crustacean identifications on the preliminary samples, (b) publish a preliminary description of the HEBBLE community (with Yingst and Fauchald), (c) supervise the sorting of the main HEBBLE samples, (d) participate in the flume studies of the shallow-water analogues.

CURRENT REPORTS AND PUBLICATIONS

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Thistle, D. 1981. "Natural physical disturbances and communities of marine soft bottoms". Mar. Ecol. Prog. Ser. 6: 223-228.

Thistle, D. 1982. "Aspects of the natural history of the harpacticoid copepods of San Diego Trough". Biol. Oceanogr. 1: 225-238 (completed with partial ONR support).

Thistle, D. In press. "The stability-time hypothesis as a predictor of diversity in deep-sea soft-bottom communities: a test". Deep-Sea Res.

ORGANISM-SEDIMENT INTERACTIONS AND THEIR INFLUENCE ON SEDIMENT TRANSPORT
IN THE BENTHIC BOUNDARY LAYER

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OBJECTIVES

To understand the role of benthic organisms in influencing sediment transport in the deep ocean by a) identifying and characterizing the biota and their spatial variability at the HEBBLE site, b) measuring sedimentary properties which influence animal abundances, distributions, and interactions with sediments, and c) evaluating these data in terms of the effect(s) of organisms on parameters of a sediment transport model.

ABSTRACT

During July, 1982, sixteen navigated 0.25 m^2 box cores were taken in a stratified random design at the HEBBLE site, a $2 \times 4 \text{ km}$ area affected by high near-bottom currents in 4800 m of water on the lower continental rise of Nova Scotia. Samples from these box cores will be used for characterization of the benthic fauna and of particular sedimentary characteristics, and their spatial variability. The July, 1982 sampling program was designed based on preliminary 0.25 m^2 box core samples taken in the same general area in April-May, 1980.

As we concluded after our first examination of this area in 1980, this deep sea region is alternately influenced by periods of strong near-bottom currents which erode and transport sediment and periods of weaker flow when sediment reworking by benthic infauna dominates particle transport. This temporal alteration of physical and biological activity is not always evident in bottom photographs but is preserved in the sedimentary fabric as observed in X-radiographs. The upper 10 to 20 cm of sediment is characterized by both physically produced small scale laminations ($\sim 1 \text{ mm}$) and larger sediment layers (3-5 cm thick), as well as extensive tube and burrow networks and localized areas homogenized by biogenic reworking. Macro- and meiofauna are most abundant in the upper 2-3 cm. On our most recent cruise, however, we were also able to document the presence of polychaete worms and sipunculids in lined borrows at depths of 20-25 cm. Most macrofauna appear to be surface deposit and suspension feeders adapted to food sources supplied by relatively strong bottom currents.

Our April, 1980 results show that macro-, meio- and bacterial numbers and ATP concentrations are lower than in nearshore sediments reflecting the limited availability of metabolizable organic matter and slow rates of net deposition in the deep sea. These densities are, however, higher than reported for other deep sea deposits of similar depth. It should be interesting to contrast our April, 1980 sampling which followed a period of strong near-bottom currents with our July, 1982 samples which were taken during an extended period of relative calm.

FUTURE PLANS

I plan to a) finish identification of bivalves and miscellaneous taxa from April, 1980 samples, b) publish a description of the general HEBBLE area macro-meiofaunal community (with Thistle and Fauchald), c) work up bacterial and sedimentological samples from the main HEBBLE sampling program, d) analyze microbiological samples taken from 17 box cores to correlate with uronic acid analyses for a measure of sediment binding-mucopolysaccharides (with White and Thistle).

CURRENT REPORTS AND PUBLICATIONS

Yingst, J.Y. 1981. Microbial distributions and sedimentologic characteristics of HEBBLE area deposits. E.O.S. 62(45):898.

Yingst, J.Y. and R.C. Aller. 1982. Biological activity and associated sedimentary structures in HEBBLE area deposits, western North Atlantic. Marine Geology 48:M7-M15.

Hollister, D.C., N.I. McCave, B.A. McKee, A.J. Silva, J.Y. Yingst. The internal structure and origin of a longitudinal triangular ripple (manuscript in preparation).

NOXIOUS MARINE ANIMALS

BIOASSAY OF SURFACTANTS AS SHARK REPELLENTS

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OBJECTIVES

1) Continue development of behavioral test methods for repellency testing; 2) test a series of 11 new substances using three bioassays previously developed in this study; 3) publish results as of 1981-1982; 4) write a comprehensive proposal which addresses laboratory and field testing of potential repellents-- and submit that proposal to ONR Oceanic Biological Program; 5) train three students under the ONR Science Education Program.

ABSTRACT

With publication of our Nav. Res. Rev. 34(2) 1982 article, we outlined progress through April 1982 in the development of behavioral test methods. During the 1982 contract period, we improved the Lethality Test by standardizing test aquaria to 150 ml and testing but a single fish (Fundulus) per trial. The Feeding Assay was improved by isolating individual sharks (Negaprion) from the group immediately prior to testing. Finally, the Tonic Immobility Assay was changed by adding a pump with $4200 \text{ l} \cdot \text{min}^{-1}$ output to the 25 l test aquarium; and giving trials by injecting test substances of known overall concentration into the pump's intake manifold. To date we have tested a total of 17 substances including: lyophilysed crude secretion of Pardachirus marmoratus; cationic, an ionic and non-ionic industrial surfactants; naturally occurring surfactants i.e. bile salts and saponin. Because of the obvious superiority of sodium and lithium lauryl sulphate, this family of well known foaming agents was studied extensively. We thus assayed lauryl-trimethyl ammonium bromide, sodium laurate, laurate ethyl ester, lauramine and lauryl alcohol. In a series of tests involving dozens of lemon sharks and hundreds of trials, we established the threshold (ranges) of responsiveness to seven surfactants.

These results provide the basis for continued experimentation on the target organ and mechanism of surfactant-induced shark repellency. We have been collaborating with D.R. Nelson on objective methods for testing chemical or other repellents in the field. Our combined efforts will focus on instrumenting the bottom of a Bahamian Lagoon with data-logging monitors and implanting longlife transmitters in a number of sharks. With this system, we will be able to predict the movements of unrestrained sharks in the absence of humans; and monitor their movements in detail. Without actually touching or seeing the sharks, we would be able to assess both the short-term (acute) and long-term (chronic) effects of repellents in the daily routine of our study colony.

Finally, three students were trained under the ONR Science Education Program. Under supervision of Mr. Allen Henningsen, the three students were located in Key Largo, Florida for the months of July and August 1982. There, they tagged and released 337 lemon sharks collected sharks by longline and gill net and studied the food habits of the captured sharks by classifying the contents of nearly 100 stomachs.

PLANS FOR THE FUTURE

We will continue to collaborate on chemical shark repellents under a 3-year Binational Science Foundation grant. In addition, we expect to collaborate with D.R. Nelson on field testing of chemical repellents. Beside determining the most effective surfactant-type repellents, we plan to determine target organ and mode of action of surfactants as repellents.

Table 1.

| Subs. | Formulae, commercial names and sources | Pupfish lethality LD ₅₀ (g.ml ⁻¹) | Feeding assay range (mg.ml ⁻¹) | Tonic immobility LD ₅₀ (mg.ml ⁻¹) |
|-------|--|--|--|--|
| 1. | Lauryl sulphate sodium | 3.0 | 0.2-2.0 | 0.45 |
| 2. | Lauryl sulphate Lithium | 6.0 | 0.2-2.0 | 0.62 |
| 3. | Pardachirus secretion | 16.0 | 0.8-3.0 | 0.66 |
| 4. | Polyethoxylated octylphenol | 36.0 | 6.0-8.0 | 8.0 |
| 5. | Lauryl trimethyl ammonium bromide | 60.0 | 3.0-8.0 | 8.0 |
| 6. | Cholic acid - sodium salt | 100.0 | 8.0-10.0 | 8.1 |
| 7. | Ethoxylated (20) sorbitan monolaurate | 100.0 | 10.0-20.0 | 10.1 |

PUBLICATIONS SUBMITTED

Gruber, S. Shark repellents: protocols for a behavioral bioassay. In: Zaharanec, B. (ed). Problems and Possibilities: The Development of an Effective Shark Repellent from Naturally Occurring Biologically Active Substances. AAAS Symposium Volume; Washington, D.C.

Zlotkin, E. and Barenholtz, Y. On the membranal action of Pardaxin. In: Zaharanec, B. (ed) *ibid*.

Zlotkin, E. and Gruber, S. A new approach to chemical shark repellents. Science (Washington).

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Gruber, S. 1981. Shark repellents: Perspectives for the future. Oceanus 24(4): 72-76.

Gruber, S. and Zlotkin, E. 1982. Bioassay of surfactants as shark repellents. Nav. Res. Rev. 34(2): 18-27.

*ONR Science education awardees.

FIELD INVESTIGATIONS OF SHARK BEHAVIOR

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NR 104-062

OBJECTIVES

(a) To investigate the behavior of sharks in the natural environment - both remotely (via telemetry) and directly (via diver observation and cine/video) - in regard to patterns of activity and movement, mechanisms of orientation and navigation, responses to attractive and aversive stimuli, feeding patterns, and intra/interspecific social behaviors including aggressive interactions with humans, and (b) to develop the biotelemetry/monitoring instrumentation and techniques necessary for the above field studies.

ABSTRACT

In September 1982, Peter Klimley completed the initial write-up of his dissertation (at SIO) on schooling hammerhead sharks. As previously mentioned, the study involved determinations of school size, sex-ratio, seasonality, including the food habits, maturity, stereotyped behavior patterns, and diel movements of individuals. Recently, an analysis was conducted on the three-dimensional structure of the schools using stereophotographic data. It was found that position in the schools and individual shark-to-shark distances can be related to size and sex.

The masters study of Jim McKibben (movements of gray reef sharks at Enewetak, Marshall Islands) was completed in summer 1982 and is now being prepared for publication. Thesis write-ups are currently in progress by Jeff Landesman (horizontal and vertical movements of blue sharks) and by Greg Pittenger (long-term movements of angel sharks). In regard to the latter, continued re-observations of tagged angel sharks are providing good information on age and growth in this species. Of 400 sharks tagged, 23 percent have now been resighted at Catalina - up to seven times, and up to two years at liberty. Measured growth ranged from about 12 cm/year (at 80 cm TL) to zero at 125 cm TL, with a growth of 3-4 cm/year at the mean size of 105 cm TL.

Field tests are underway to determine the effects of "repellent" chemicals on bait-attracted local pelagic sharks, e.g., blue and mako. This work is being coordinated with the laboratory assays of Gruber and Zlotkin at the University of Miami. Initial tests in California will utilize inexpensive, readily available substances (surfactants, household ammonia, etc.), and are aimed at developing workable procedures for the quantitative field testing of candidate repellent substances including those of natural origin. Preliminary trials with the surfactant sodium dodecyl sulfate (sodium lauryl sulfate) have been quite positive.

Direct injection of SDS into the oral cavity of blue sharks (while feeding on bait) can have a dramatic repellent effect. About 100 cc of a 15% solution causes immediate rejection of the bait and a rapid withdrawal with mouth held wide open. A 1% solution delivered in this manner also repels, but less dramatically, as does delivery underwater by divers using a squirt applicator. Besides the screening of potentially repellent substances, plans call for studies of the orientation responses of sharks in aversive chemical fields.

We have started developing a new series of ultrasonic telemetry monitors to be used for automated, long-term, continuous tracking of telemetered sharks. Each monitor unit will have a two-frequency narrow-band sonic receiver and a micro-processor programmed to recognize and verify at least 32 individual transmitter codes. The appearance and disappearance of valid codes will be stored in low-power RAM, along with the time of day to the nearest minute. The units will be able to recognize at least several codes simultaneously, and will have sufficient power for a duration of up to one month between servicings (data extractions). The system will be initially tested on angel sharks at Catalina Island, California. Eventually, it is planned to build 20 or 30 monitors for a study (with S.H. Gruber, University of Miami) of lemon sharks in Bimini, Bahamas. One objective of this work is to determine if established home areas for certain sharks can be altered by presenting low concentrations of "area repellent" chemicals.

Besides our present 26' boat, we will soon have available a 48' twin-diesel research vessel that is now being built. This larger vessel will accomodate more personnel and instrumentation for longer periods of time, and will be used for overnight and multiday operations such as longer-term pelagic trackings.

PLANS FOR FUTURE

(a) To continue field studies of shark behavior and ecology, including tests of potential repellent chemicals, on blue, mako, angel, and great white sharks in California, gray reef sharks in the tropical Indo-Pacific, hammerhead sharks in the Gulf of California, and lemon sharks in the Bahamas, and (2) to continue development, as needed, of instrumentation including ultrasonic transmitters, radio float transmitters, X-Y positioning systems, and unmanned telemetry monitors.

CURRENT REPORTS AND PUBLICATIONS

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- Klimley, A.P. 1981. Grouping behavior of the scalloped hammerhead. Oceanus 24(4): 65-71.
- Nelson, D.R. 1981. Aggression in sharks: Is the gray reef shark different? Oceanus 24(4): 45-55.
- McKibben, J.N. 1982. A telemetric study of the movements of gray reef sharks, Carcharhinus amblyrhynchos, at Enewetak Atoll, Marshall Islands. Masters thesis, CSULB.
- Nelson, D.R. (in press) Shark attack and repellency research: An overview. In Zahuranec, B.J., ed., Shark Repellents from the Sea: New Perspectives. AAAS.
- Klimley, A.P. (in ms) Social organization of the scalloped hammerhead shark, Sphyrna lewini, in the Gulf of California. Ph.D. Dissertation. UCSD (SI0).
- Klimley, A.P. and D.R. Nelson. (in ms) Diel movement patterns of scalloped hammerhead sharks, Sphyrna lewini, at El Bajo Espiritu Santo, and their relation to the refuging concept.
- Klimley, A.P. (in ms) School composition and structure, and its origins, in scalloped hammerhead sharks, Sphyrna lewini.

BIODETERIORATION

MECHANISMS GOVERNING INDUCTION AND INHIBITION OF SETTLING AND
ESTABLISHMENT IN SOME SESSILE AND BENTHIC MARINE ORGANISMS

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OBJECTIVES

The broad-scope objective of this project is comparison of biochemical pathways regulating induction or inhibition of settling and colonization in cirriped and decapod crustaceans and in certain seaweeds in culture. Objectives specified for two subprojects are: 1) Studies on endocrine and neurosecretory systems and their functions in site selection, settling and metamorphosis in several species of crabs and Balanus species. 2) Establishment in culture of: (a) certain secondarily reduced epiphytic red seaweeds; (b) several genera of red seaweeds serving as hosts for parasitic red algae; (c) the parasitic species themselves -- for the purpose of carrying out morphological and cytochemical studies relative to spore settling and germination. Subsequent objectives will be isolation, classification and ultimate identification of substances inducing or inhibiting spore germination and colonization.

ABSTRACT

Studies were concluded on the role of temperature in glycogen deposition relative to pre-molt conditions and metamorphosis in xanthid mud crabs (Rhithropanopeus harrisii). Lawrence Williams, the graduate student involved in this project, is writing his thesis now based upon this work.

A portion of our work necessarily centers on manipulating culture conditions, using recirculating real and artificial seawater mixtures, until appropriate for Balanus species, Menippe mercenaria and other local crustacean species. Accumulation of culture facilities with auxiliary air conditioning and ambient cool water circulation designed to circumvent chronic problems arising from defective central air conditioning will enable us to accomplish most of our thus far postponed objectives over the next few months.

A new culture chamber allowed seaweed work to proceed: five genera of host plants are in culture, which will be infected with spores from parasitic genera scheduled to arrive in early October. At present, one genus of reduced epiphytic red seaweed is in culture. Large amounts of variously fixed material of this latter genus is ready for sectioning and completion of preliminary TEM studies on cell walls thus far unique to the tribe Herposiphoniae (secondarily reduced red seaweeds in the family Rhodomelaceae). A certain amount of morphological work is being done to support the main purpose of investigating mechanisms governing seaweed spore settling and germination.

Some red seaweed hosts appear to produce, not the expected inhibitors of parasitic growth, but substances that induce germination of parasitic spores on host plants. In most red marine algae, parasites will germinate only on closely related hosts. This circumstance is particularly intriguing when compared to the opposite condition that prevails in epiphytic red algae, which germinate and thrive to adulthood on almost any algae except those that are very closely related to the epiphytic genus. Cytochemical activity in these opposing situations, if identified, could lead to answers to questions relating to biofouling, parasitism and immunology in genera. Surface conditions, including microflora and their relevance to successful settling and germination of spores, will constitute part of this study.

PLANS FOR THE FUTURE

- a) Conditions, organisms and production of biochemicals contributing to site selection in barnacles will be examined along with endocrine and neurosecretory products related to molting and metamorphosis in barnacles and Menippe.
- b) Ultrastructure and epifluorescence studies relative to cytogenetics of reduced epiphytic and parasitic red algae in the Rhodomelaceae will be undertaken.
- c) Immuno-cytochemistry of parasite/host associations related to settling and subsequent germination of tetraspores, bispores and carpospores of rhodomelacean parasites will be a major emphasis.
- d) Consideration will be given to the feasibility of culturing some coralline algae, particularly comparing hosts and parasitic species, in order to investigate metabolism and deposition of calcium by spores during germination.

CURRENT REPORTS AND PUBLICATIONS

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- (b) Williams, L. and V.G. Archer. 1982. Glycogenesis in Rhitropanopeus harrisi larvae under conditions of constant compared to cyclical temperatures in culture. MS Thesis, Jackson State University. In prep.

INVESTIGATION OF THE INITIAL EVENTS IN BIOFOULING

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NR104-17.2/6-19-81

OBJECTIVES

(a) To document the rate, sequence, and consequences of the earliest micro-fouling events on synthetic/engineering substrata exposed to seawater and brackish water environments, (b) to relate these events to controllable properties of the substrata, such as their relative surface energies, and to colonization of the primary adsorbed films by pioneer microorganisms, (c) to determine the prospects for shear-induced detachment of the biofouling layers from controlled substrata devoid of all toxicants, and (d) to support the biofouling investigations of other research teams by providing surface-characterized substrata and surface chemical/physical characterization of the microfouling layers predisposing to settlement/adhesion of macrofouling species.

ABSTRACT

A 100-gallon synthetic seawater aquarium network, with an integral algal scrubbing tank, has been set up with a complete fouling population derived from macrofouled panels. Novel flow cells of three types (isothermal, thermal-chambered, and heat-exchange measurement) have been constructed and operated in both the aquarium system and natural seawater/brackish water systems to demonstrate the adequacy of the model system as a simulant for natural events. The sequence of microfouling events was shown to be identical among the systems tested, beginning with rapid adsorption of a protein-dominated "conditioning" film, then colonization by rod-shaped bacteria, secretion of polysaccharide exudates, secondary colonization by prosthe-cate microorganisms and their secretion and replication, elaboration of the fila-mentous appendages and trapping of inorganic debris. Algal attachment and growth seems to be a later event, as does attachment of the larval forms of macrofoulers. The rate of these events in the laboratory aquaria is suppressed ten to 100-fold over that in once-through natural waters.

Microbiological examination of substrata exposed in the aquaria demonstrated populations similar to those of the natural waters tested. Reflected light microsc-opy has been shown to be useful in examining the earliest attached organisms in situ on opaque, reflective test plates, and immunofluorescence techniques are now being developed (along the lines already successful for oral microorganisms) to aid in speciation. Examination of substrata of surface energies varying from very low (characteristic of fluorocarbons like Teflon) to very high (characteristic of scrup-

ulously clean metals, glasses, and ceramics) has shown differences in organism abundance and type, but considerable additional work remains to confirm and extend these findings. Glass substrata have been coated with very thin (less than 200 Å) layers of surface-energy-control substances and multiple specimens of 5 different surface energy states have been sent to other investigators for exposure to fouling environments of great diversity. Specimens have been returned from Kevin Marshall (Australia) after exposure to intrinsically hydrophobic and intrinsically hydrophilic strains of fouling bacteria, and examination of the colonization success is in progress of each organism type on each substratum. H. Waite (Connecticut) has returned specimens exposed to mussel fouling environments, and these are similarly under scrutiny at present. Keith Cooksey (Miami/Montana) has completed an experimental series with diatom adhesion and produced a "relative attachment" curve for these surface-controlled substrata that faithfully mimics the curve previously developed for human blood platelet binding to the same materials. Additional experiments are in progress elsewhere, with samples to arrive for analysis shortly.

Work in both the laboratory and in brackish waters of the Hudson River with a novel heat-exchange flow cell has shown apparent sensitivity of the new apparatus to microfouling events within the so-called "induction period" not accessible to measurement by prior devices. Application of surface chemical/physical methodology to the flow cell test plates is now allowing correlation of the heat transfer changes with specific fouling events, and the prospect emerges that different heat transfer transients may be developed as signatures for fouling of differing organic/inorganic ratios.

PLANS FOR THE FUTURE

(a) To modify the flow cells by substituting one transparent surface-controlled glass plate for one of the usually opaque metallic specimens, to allow for adequate illumination and consequent development of algal fouling simultaneous with bacterial microfouling, (b) to isolate, culture, and identify specific organisms from the primary microbial layer and generate immunofluorescent stains able to provide in situ speciation of organisms still attached to the fouled substrata, (c) to instrument the flow cells with pressure transducers suitable for monitoring shear-induced detachment and/or drag coefficients of microfouling layers on substrata of controlled surface properties, and (d) to provide germanium internal reflection prisms to collaborating investigators for immersion in diverse fouling media and return for surface chemical/physical characterization of "conditioning" films that precede organism attachment in their test systems.

CURRENT REPORTS AND PUBLICATIONS

- Baier, R. E. "Initial Events in Microbial Film Formation," Proceedings, Symposium on Marine Biodeterioration, U. S. Naval Institute, in press.
- Baier, R.E., DePalma, V.A., Meyer, A.E., King, R.W., and Fornalik, M.S. "Heat Exchange Surface Microfouling During the Induction Period." Journal of Heat Transfer, accepted for publication subject to minor modifications in accord with reviewer's comments.
- Baier, R.E. "Conditioning Surfaces to Suit the Biomedical Environment: Recent Progress," review article for Journal of Biomechanical Engineering, in press.

MICROBIAL FOULING AND ITS EFFECT ON POWER GENERATION

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NO014-82-c-0475

NR104

OBJECTIVES

(a) Develop a unified theory which describes biofilm development and the resulting energy losses in heat exchange equipment, (b) investigate the effect of particulate inclusions and inorganic constituents within the biofilm on biofilm structure and energy losses, (c) develop methods for measuring the extent of biofouling, its effect on energy losses, and the effectiveness of biofilm destruction processes, (d) determine the effectiveness of selected chemical methods for biofouling control.

ABSTRACT

Biofilms develop on the tube side (i.e., cooling water side) of heat exchanger/condenser surfaces increasing fluid frictional resistance and heat transfer resistance. Biofilm accumulation leads to increased steam pressure on the shell side (i.e., the steam side) of the condenser and reduces turbine efficiency. Consequently, more fuel is consumed per unit power produced. Increases in fluid frictional resistance also lead to increased power consumption for pumping. The resulting effects of biofouling in ship systems is increased energy consumption for normal operation and a decrease in available peak power.

This project was initiated on 15 May 1980. Since that time, we have accomplished the following:

- (1) Structured mathematical models have been developed to describe biofilm growth and the effect of biofilm on heat transfer resistance. A suitable expression has been developed which describes biofilm detachment rate as a function of biofilm mass. Significant progress has been made on modelling and experimental methods to determine effects of transient conditions on biofilm process.
- (2) Three different experimental systems have been constructed and are being used to study biofilm development, the interaction of biofilm with inorganic constituents, the effectiveness of various chemical methods for biofouling control, and the interaction of biofilms with the corrosion process.
- (3) Analytical techniques have been developed for our continuing work with defined and mixed bacterial cultures. A chemostat system which provides a continuous supply of mixed bacterial culture has been successfully used to provide bacteria to a simulated heat exchanger.

(4) Biofilm development with S. natans and Ps. aeruginosa has been studied and compared with particular attention to biofilm morphology and the effect of frictional resistance. Kinetic expressions describing the growth and product formation of Ps. aeruginosa in a dispersed cell chemostat have been determined and compared to initial results of Ps. aeruginosa grown in an attached biofilm system.

(5) The effectiveness of the following methods for biofouling control has been determined: chlorine, the Cathelco system, hydrogen peroxide, and copper-nickel and titanium, have been compared for their ability to resist biofouling.

(6) Experiments have been conducted to determine the relationship between biofouling and scaling.

(7) A workshop, "Fouling of Shipboard Heat Transfer Surfaces", was held in Bozeman, MT 23-25 March, 1982 sponsored by the Power Program, ONR.

PLANS FOR THE FUTURE

(1) Examine the effects of oxygen concentration on biofouling and corrosion of copper-nickel heat exchanger tubing, (2) investigate the relationship between corrosion and biofouling at the molecular level, (3) determine the effects of fluid shear stress and influent organic concentration on scaling and biofouling of copper-nickel and stainless steel, (4) examine the role of extracellular polymers in the microbial physiology and ecology of attached microbial cells. (5) evaluate factors influencing transport of microbial cells to surfaces and the reversible/irreversible adhesion to the surface.

CURRENT REPORTS AND PUBLICATIONS (1981-1982)

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Zolver, N., Flandreau, J. R., Spataro, W. H., Chapple, K. R., Characklis, W. G., and Roe, F. L., "Analysis and Monitoring of Heat Transfer Tube Fouling." Proceedings, 1982 Joint Power Generation Conference, Denver, Oct. 1982.

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Characklis, W. G., Zolver, N., Dobb, D. E. and Nelson, C. R., "Influence of Biofouling and Biofouling Control Techniques on Corrosion of Copper-Nikel Tubes." Proceedings, Symposium on Corrosion Induced by Bacteria, CORROSION/83, Anaheim, CA April 1983.

Presentations at Technical Meetings (* Invited)

*Zolver, N., Legan, R. and Characklis, W. G., "Biofouling Control with UV/Peroxide: A Laboratory Study." AWWA Water Reuse Symposium II, Washington, D. C. (August 1981).

*Characklis, W. G., "Biofilm Formation: Effects and Control." Seminar "Biotechnology in the Pulp and Paper Industry," Pulp and Paper Research Institute of Canada, Pointe Claire, P.Q., Canada (Sept. 1981).

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*Bakke, R., Characklis, W. G., and Trulear, M. G., "A Comparative Evaluation of Substrate Utilization by Attached and Suspended Biomass." A.I.Ch.E. Annual Meeting, Symposium on Fixed-Film Systems for Industrial Wastewater Treatment, New Orleans, LA (Nov. 1981).

Characklis, W. G., Trulear, M. G., and Bakke, R., "Biofilm Processes." ACS/IEC Winter Symposium, Foundations of Biochemical Engineering: Kinetics and Thermodynamics in Biological Systems, Boulder, CO (Jan. 1982).

Matson, J. B. and Characklis, W. G., "Biofouling Control in Recycled Cooling Water with Bromo Chloro Dimethylhydantoin." Cooling Tower Institute Annual Technical Meeting, Houston, TX (Jan. 1982).

*Characklis, W. G., "Engineering the Microbe's Environment: Biodegradation." at Impact of Applied Genetics in Pollution Control, University of Notre Dame, (May 1982).

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A PHYSIOLOGICAL INVESTIGATION OF THE ADHESION OF
DIATOMS TO SURFACES

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NO 004-80-0042

NR 083-060

OBJECTIVES

(a) To investigate the means by which fouling diatoms attach to submerged surfaces. (b) to analyze biochemically the attachment polymer (adhesive) produced by diatoms, (c) to demonstrate potential interactions between bacteria and diatoms mediated by organic materials secreted by the diatoms.

ABSTRACT

Amphora coffeaeformis, a benthic marine diatom is found commonly on surfaces treated with antifoulants. Previously we have demonstrated the Ca-dependence of attachment in this organism. Studies of the adhesive properties of this organism have continued in our laboratory using a microbiological adhesion assay. The assay has been improved to the extent that 50% (S.D. = 5.0, n = 24) of the cells added to the assay vessels adhere to the test surface. This has facilitated our use of drugs which are known to perturb certain phases of cellular adhesion in other systems. We have investigated the action of polysaccharidases, podophyllotoxin, various cytochalasins, tunicamycin and cycloheximide. Neuraminidase, hyaluronidase and mannosidase did not inhibit adhesion, but dihydrocytochalasin B (DHCB - an inhibitor of the assembly of actin cytoskeletal microfilaments) podophyllotoxin (PDT - an inhibitor of microtubule synthesis), tunicamycin (TUN - an inhibitor of protein glycosylation in mucopolymer synthesis) and cycloheximide (CH - an inhibitor of eucaryote cytoplasmic protein synthesis and in some cases Ca - membrane interactions) did. The 50% effective concentrations were in the 50-100 μ M range for DHCB and DDT, but considerably less (1-3 μ M) for CH and TUN. PDT, DHCB and CH were found not to be cytotoxic at the concentrations used. TUN has not been tested yet. In other experiments using the adhesion assay, we have shown that attachment of diatoms to glass surfaces is not particularly pH-dependent. At pH 6.7, attachment is 70% of that at pH 7.7. This has allowed us to investigate the effects of La^{3+} on adhesion since La is not very soluble at pH 7.7. 1mM La^{3+} inhibits adhesion 90%; higher concentrations cause cell plasmolysis. After pre-conditioning glass microscope cover-glasses in medium in which diatoms had previously adhered, we were unable to detect a difference in the attachment kinetics of a further population of A. coffeaeformis cells. This is not to say that the formation of a preconditioning film is not involved in this attachment, but that we could not detect its involvement.

We now have a method for measuring ^{45}Ca influx in A. coffeaeformis. So far we have been able to show that while formaldehyde-treated cells do not accumulate ^{45}Ca

from a 5 mM Ca solution, whereas cells treated with the Ca-blocking agent D-600 accumulate about half the calcium of untreated controls.

Efforts to show that there is potentially an exchange of organic materials between attached diatoms and bacteria have been successful. Acid-stable ¹⁴C-labelled materials from the diatoms *A. coffeaeformis*, a small *Navicula* sp. and *A. coffeaeformis* var. *perpusilla* were prepared and their uptake by a marine *Vibrio* isolated by Gerchacov and Udey (Strain B27-11A), was measured. Diatoms secreted 5-10% of the label added to the cells and approximately one sixth of this material was assimilated by the bacteria in 20 minutes. After this time radioactivity in the bacterial cells declined, probably a result of respiration. The initial production of the labelled materials was carried out in such a way that diatom cell lysis and subsequent release of organic materials was highly unlikely. These experiments show the potential for an autotroph-heterotroph interaction in a fouling community is considerable.

Our results with anticytoskeletal drugs and inhibitors of Ca transport are consistent with the hypothesis that vesicles (seen in T.E.M) containing adhesive polymer are transported to the raphe canal via a microtubule system. The synthesis of the adhesive, which could take place in the dictyosome region of the cell, may be sensitive to tunicamycin. On reaching the canal, the vesicles undergo a Ca-dependent exocytosis leading to the attachment of the diatom cell to its substrate at the raphe canal.

PLANS FOR FUTURE

(a) We expect in the future to be able to undertake biochemical analysis of the adhesive polymer. This will be attempted in collaboration with Dr. R. Baier of Calspan Corporation of Buffalo, N.Y. and Dr. D. White of Florida State University, Tallahassee, Florida (b) attachment of diatoms to surfaces of differing surface energies will also be investigated. (c) we hope to gather information in support of the preliminary model of diatom adhesion elaborated above.

CURRENT REPORTS AND PAPERS

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Division of Colloid Science, American Chemical Society, in symposium on 'Early Events in Bioattachment.' March, 1982, Las Vegas, NV. (To be published in Advances in Chemistry of A. C. S.).

A similar paper was given at a workshop on 'Fouling of Shipboard Heat Transfer Surfaces' sponsored by the Office of Naval Research, March, 1982 at Montana State University, Bozeman, MT.

STUDIES ON MOLTING AND GROWTH IN LARVAL AND ADULT BARNACLES AND LARVAL DECAPODS

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104-194

OBJECTIVES

(a) To further explore the occurrence and composition of naturally occurring barnacle repellents and attractants found in soft corals and (b) the role of endocrine mechanisms in controlling seasonal cycles of gametogenesis in Balanus and (c) to determine if cement synthesis and release in adult barnacles is regulated by molting hormones.

ABSTRACT

Using cyprid larvae of several species of Balanus as a bioassay, varying concentrations of Leptogorgia extracts have been tested to determine the degree to which various components of the extract will attract or repel settlement and metamorphosis to the pinhead stage. Recent data indicate that the molecular size of the active ingredient is less than 10,000 daltons. At various concentrations the extract inhibits settlement of barnacles, a true inhibitory effect rather than a toxic one. There are indications that the concentration of the inhibitory substance in the coralline tissues may vary seasonally and at least one other soft coral, Renilla, produces a substance which also inhibits barnacle settlement.

Extracts from the central nervous system of several species of Balanus, when injected into adult barnacles, results in modifications in the size of oocytes, ovarian tubules, and seminal vesicles as well as the proportions of the various germ cell types within the ovary. The central nervous system extracts from winter field animals inhibit development of both male and female gonads. Similar extracts simulate growth of the gonads. These studies indicate that an antagonistic pair of neurohormonal factors is responsible for the yearly onset and termination of gametogenesis.

Multiple injections of molt inhibiting hormone (MIH) into adult barnacles produced a variety of effects on cement production at different stages within the molt cycle. MIH-injected adults showed a decreased cement production at a time when MIH would not normally be found in the barnacles.

PLANS FOR FUTURE

(a) To identify the attractants and repellents in soft corals and determine the way in which barnacle cyprids respond to these substances at the time of settling and metamorphosis; (b) to determine the way in which neurosecretory cells within the central nervous system may control metamorphosis and cement synthesis and release in recently settled pinhead barnacles.

CURRENT REPORTS AND PUBLICATIONS

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- Tomaszewski, C. and J.D. Costlow. Effects of estradiol-17 on oogenesis in Balanus amphitrite. In preparation.
- Standing, J.D., I.R. Hooper and J.D. Costlow. Inhibition and induction of barnacle settlement by natural products present in octocorals. In preparation.
- Freeman, J.S. and J.D. Costlow. The cyprid molt cycle of the barnacle, Balanus amphitrite. In preparation.
- Freeman, J.S. and J.D. Costlow. Endocrine regulation of premolt in Balanus amphitrite cyprids. In preparation.
- Freeman, J.S. and J.D. Costlow. Endocrine control of spine epidermal resorption during metamorphosis in Rhithropanopeus harrisii. In preparation.
- Freeman, J.S. and J.D. Costlow. Post-metamorphosis molt cycle regulation in juveniles of the crab, Rhithropanopeus harrisii. In preparation.
- Freeman, J.S. and J.D. Costlow. Spine regeneration in larvae of the crab Rhithropanopeus harrisii. In preparation.
- Freeman, J.S. and J.D. Costlow. Crustacean growth and metamorphosis interaction of endocrines and the epidermis. In preparation.

NON-POLLUTING WOOD PRESERVATION

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N00014-82-WR-20215

NR 104-767

OBJECTIVES

To develop new natural and synthetic wood preservatives that can be made available to the Navy in the event that present day wood preservatives (arsenicals, creosote, and pentachlorophenol) are prohibited.

ABSTRACT

A cooperative research program to develop and screen promising new products and techniques for preserving wooden piers and pilings is being conducted by a consortium of wood-user countries. So far our search for new naturally resistant wood has uncovered two promising species; Latesta durrissima and Baillonella totisperma are both resistant to all marine wood borers and fungi that we have exposed them to. Several other timbers have shown selective resistance to certain other wood borer species.

Two non-arsenical wood treatments (copper-chrome-boron and copper-chrome-phosphorous) are being field evaluated in Panama. The 6% and 10% retentions in wooden test boards are resisting attack by borers and fungi after 8 years.

A study is presently underway to determine if microorganisms can selectively detoxify chemical wood preservatives. We have found, for instance, that wood treatment durability varies from place to place, often unrelated to the abundance or kinds of wood borers, or obvious physical environmental differences.

We are also testing a heat-shrinkable teredo barrier for long term durability. This new material can be quickly and tightly applied to pilings before driving, and can be repaired in the water. After three years in Panama, test pieces remain undamaged.

FUTURE PLANS

The long term exposures are continuing. We will begin testing polyolephin heat-shrinkable pile wraps at marine test stations in the North Sea, Eastern Mediterranean Sea, Arabian Sea, and western South Pacific Ocean, to certify worldwide durability. We will also begin evaluating its resistance to termite attack at the Army Tropical Test Center in Panama.

CURRENT REPORTS AND PUBLICATIONS

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Bultman, J. D., E. C. Haderlie, J. R. DePalma. 1980. Comparative resistance of four Central American hardwoods to marine borer attack. Proc. 4th Int'l Biod. Sym. pp. 199-203. Editors: T. A. Hoxley, G. Becker, D. Allsopp. The Biodeterioration Society, London, England.

THE EFFECTS OF ORGANIC MATTER AND SURFACE ASSOCIATED MICROORGANISMS ON METAL CORROSION IN THE MARINE ENVIRONMENT

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N00014-76-C-0076

NR 083-300

OBJECTIVES

(a) To determine the growth behavior of a marine *Pseudomonas* sp. (strain B-3), particularly the production of intracellular red spherical particles by this organism; (b) to determine the effect of this highly copper-resistant pseudomonad on the corrosion of copper in seawater.

ABSTRACT

Strain B-3 turns red under some growth conditions, but only in the presence of copper and either glutamic acid or mixed amino acids. This red color is associated with the accumulation of small (0.1 μ m) spherical particles associated with the peptidoglycan layer of the cell wall. The production of these particles is inhibited during aerobic growth, and only appear when the cultures are shifted to microaerophilic conditions. This pattern was repeatable at several growth rates, controlled in a chemostat. A partial purification of these particles has been achieved.

Strain B-3 was inoculated into our flow-through corrosion cells with glutamic acid and glucose as sole carbon sources. This strain is one of the few in our collection which grows well in association with copper when grown on glucose as the sole carbon source. Although it does not accumulate the red-spheres when growing on glucose, it does appear to deposit highly refractile copper-containing material in or near the cell wall.

Corrosion rate measurements for copper in the flow-through cells were continued following inoculation with bacteria. The results of these measurements, which are still being taken as a function of time, were compared to the internal baselines previously obtained. These measurements indicate that bacterial strain B-3 increased the corrosion rate of copper in the presence of glucose as a sole carbon source. This increase was accompanied by increased admittance (decreased polarization resistance), decreased cathodic Tafel slope, and an anodic shift of the corrosion potential values. In glutamic acid augmented seawater, strain B-3 decreased the corrosion rate of copper. This change was accompanied by an increased polarization resistance, decreased cathodic Tafel slope, and a cathodic shift of the corrosion potential values. The decrease in cathodic Tafel slopes in both glucose and glutamic acid suggests that the presence of B-3 either removed the barrier to oxygen diffusion to the corroding surface, or changed the primary cathodic reaction making the presence of oxygen at the corroding surface unnecessary.

The copper specimens in the flow-through cells will be examined by scanning electron

microscopy to determine the uniformity of bacterial surfaces coverage, and metal corrosion. X-ray diffraction analysis will be made to identify corrosion products on the surfaces.

Attempts to grow a Micrococcus sp. originally isolated from brass surfaces exposed to seawater, on copper in seawater has repeatedly failed. This strain appears to be too sensitive to copper to colonize the surface of a corroding copper electrode. Co-cultures of this organism with B-3 in the presence of copper strips resulted only in the attachment and growth of B-3, i.e., B-3 could not "detoxify" the copper sufficiently to allow growth of a more sensitive strain.

An attempt was made to enhance the attachment of the Micrococcus to other surfaces, such as glass and titanium, by "conditioning" the surface with other efficiently attaching bacteria, or their culture supernatants. None of the pretreatments improved attachment of the Micrococcus. These strains of Micrococcus may quickly loose their ability to attach to surfaces once cultured on bacteriological media.

PLANS FOR THE FUTURE

(a) Investigate the mechanism by which pseudomonads B-3 and CN-50 affect the corrosion of copper in seawater, (b) investigate the relationship of bacterial and substrata surface properties to the attachment of bacteria.

CURRENT REPORTS AND PUBLICATIONS

Gerchakov, S.M., L.R. Udey, and F. Mansfeld. 1981. "An improved method for analysis of polarization resistance data". Corrosion, 37:696-700.

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BIOLOGY OF STONE AND WOOD BORERS IN MONTEREY SUBMARINE CANYON
AND DEEPER WATER OFF CENTRAL CALIFORNIA

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N0001481WR10016

NR083-275-o8

OBJECTIVES

(a) To determine the horizontal and vertical distribution of stone and wood boring animals in the deeper waters of Monterey Bay and the Monterey Submarine Canyon, (b) by using experimental panels of wood, stone and concrete placed at various depths to determine settling time, rates of growth, and longevity of borers, (c) to experimentally monitor the growth of stone borers by placing them in artificial burrows in stone panels then follow growth through x-ray and acoustic techniques, and (d) to learn, if possible, the mechanisms used by bivalves in penetrating stone.

ABSTRACT

During the past year we have extended our studies to depths greater than 600m and have confirmed that the wood borer Xylophaga washingtona attacks all species of wood tested at all depths beyond 75m. Many of our release mechanisms failed, but in successful recoveries from 1000m depth Xylophaga destroyed untreated wood (oak, pine, fir and redwood) after 6 months exposure. The shipworm Bankia setacea was not found below 75m depth. No stone borers penetrated any of the samples exposed below depths of 50m.

Radiographic studies on growth rates of several species of stone borers have continued. We now have good data on growth rates of several species of pholads, but mytilids such as Lithophaga still will not grow in artificial burrows. We have been unsuccessful so far in recording acoustic signals one would expect if the stone was being bored by mechanical means. In collaboration with electrophysiologists from Woods Hole Oceanographic Institute we are now attempting to correlate stone excavation with adductor muscle activity in pholads.

PLANS FOR FUTURE

(a) During the coming year distributional studies will continue but emphasis will be placed on studying boring mechanisms. Using a variety of techniques we hope to rule out or in the possibility that pholads penetrate hard siliceous substrate by mechanical means. (b) In cooperation with scientists from Scripps Institution of Oceanography we will continue studying the early developmental stages and general biology of Xylophaga.

CURRENT PUBLICATIONS

Haderlie, E. C. (in press) Monitoring growth rates in wood and rock boring bivalves using radiographic techniques. Proceedings of the Fifth International Biodeterioration Symposium (to be published Fall, 1982)

Haderlie, E. C. (in press) Long-term natural resistance of some Central American hardwoods to attacks by the shipworm Bankia setacea (Tryon) and the gribble Limnoria quadripunctata Holthius in Monterey harbor. The Veliger (to be published January 1983)

Haderlie, E. C. (in press) Depth distribution and settlement times of the molluscan wood borers Bankia setacea (Tryon, 1863) and Xylophaga washingtona Bartsch, 1921, in Monterey Bay. The Veliger (to be published April 1983)

BIOENERGETICS OF WOOD BORING MOLLUSCS

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OBJECTIVES

The marine wood boring molluscs (shipworms) are unique amongst marine bivalves in that they (a) are the only family to exhibit the complete range of reproductive patterns from broadcast spawning (ovipary) to full term larval brooding (larvipary) and (b) bore into and, in the case of the Teredinidae, ingest and digest wood in the marine environment. Our research is intended to describe the interrelationship of reproductive activity, growth, and nutritional status in shipworms through a comparative study of the gross energy budgets and changes in biochemical composition of selected species throughout their life cycle.

ABSTRACT

We have completed gross biochemical analysis of larvae of Bankia gouldi and Teredo navalis grown in 50-200 L volume cultures under optimal food and temperature conditions, and following short intervals of starvation and during delay of metamorphosis. B. gouldi larvae exhibit an increase in mean dry weight per larva from 0.058 μg to 2.86 μg during the 40 days following first appearance of the shelled larvae (ash free dry weights 0.028 and 1.181 μg respectively). Increments in protein and lipid content during this period were from 9.7 ng and 2.8 ng/larvae to 674 and 214 ng/larva respectively. Both lipid and protein are utilized extensively as respiratory substrates as indicated by loss of these components during starvation stress (e.g., a 19 day old larva loses 14.59 ng or 66% of its lipid and 42.7 ng or 48% of its protein content during a four day starvation period. Together these account for 95.5% of total caloric loss on starvation.) Similar data were obtained for the larvae of T. navalis with an increment of dry and ash free dry weight respectively from 0.292 and 0.047 μg respectively at first release to 2.12 and 0.807 μg respectively at 30 days post release. 94.5% of total caloric loss under starvation stress at 18 days old is accounted for by lipid and protein catabolism. Estimates of caloric losses made from gross biochemical composition agree well with independent estimates from respirometry. Data sets for M. cuneiformis show similar trends. Estimates of assimilation efficiency of B. gouldi larvae feeding upon the flagellate Isochrysis galbana were obtained by the ^{14}C method and found to decrease both with increasing food concentration and with increasing age of larva: all values were in the range 30-60%.

Biochemical analysis of post metamorphic B. gouldi and T. navalis grown in either one micron filtered seawater or with a supplement of I. galbana have been completed. No enhancement of growth is evident in phytoplankton supplemented

regimes; however, a consistent decrease in mean carbohydrate content, expressed as a percentage of ash free dry weight, is notable in both species (5.0% versus 28.0% for *B. gouldi*, 12.0% versus 45.7% for *T. navalis*) as is an increase in lipid content (8.2% versus 3.5% for *B. gouldi*, 7.2% versus 3.5% for *T. navalis*). We attribute these differences to a marked increase in the lipid content of the diet with phytoplankton addition.

We have investigated net uptake of dissolved amino acids by the whole adults, isolated gill, isolated mantle and larval forms of *T. navalis*, *B. gouldi* and *Lyrodus pedicellatus*. Uptake rates for isolated components are comparable to values obtained for gill or mantle of other marine bivalves (*Crassostrea virginica*, *Mercenaria mercenaria*, *Mytilus edulis*, *Argopecten irradians*, *Modiolus demissus* and *Mya arenaria*). We have assayed the activity of glutamate dehydrogenase (G.D.H.) in *B. gouldi*, *T. navalis* and *L. pedicellatus* adults. Activities were consistently 10-50 times higher than *Mytilus edulis*. Higher enzyme activities were obtained with NADPH rather than NADH as an energy substrate suggesting that the enzyme may, in part be of bacterial origin. Symbiotic bacteria isolated from the Gland of Deshayes of the above shipworms and cultured by Dr. J. Waterbury (WHOI) also show elevated NADPH specific GDH activities. Calculations based upon observed uptake rates of dissolved amino acid and ammonia, and nitrogen content of adult specimens suggest that "dietary" nitrogen requirements could be satisfied from these sources alone.

FUTURE PLANS

Completion of (1) ^{14}C assimilation studies with larval and adult forms of *T. navalis*, *B. gouldi* and *M. cuneiformis*, (2) post metamorphic physiological recordings of above species, (3) publication of material as listed below.

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THE ROLE OF MICROORGANISMS IN
MARINE FOULING AND BORING PROCESSES

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OBJECTIVES

(a) To identify the specific developmental cues, produced by bacteria in surface films, that induce the settlement of marine fouling larvae. Our data suggest that the biochemical cues are carbohydrates, which are associated with the surfaces upon which settlement occurs; (b) to characterize the mechanism by which fouling larvae recognize the settlement cues produced by bacterial films, and thereby select a surface on which to settle and metamorphose. Our data suggest that the larvae possess surface proteins that bind specifically to carbohydrates present in certain bacterial films on submerged surfaces; and (c) to determine whether biochemical cues produced by bacteria induce the settlement of other important invertebrate and algal fouling organisms.

ABSTRACT

Our research is intended to provide a better understanding of the interactions that occur between microorganisms and marine fouling and boring invertebrates. Recently, our studies have sought to define the role that microorganisms play in invertebrate settlement processes.

Bacteria appear to be important in the larval settlement and metamorphosis of many marine invertebrates. In 1980 we demonstrated that larvae of Janua (Dexiospira) brasiliensis settle and metamorphose on films of bacteria.

It appears that lectins (proteins that bind to specific carbohydrates) on the larval surface bind to extracellular polysaccharides produced by certain bacterial species; this binding constitutes a biochemical recognition that induces a larva to settle and begin metamorphosis.

Our data suggest that larval settlement and metamorphosis of Janua is a lectin-mediated process. Our hypothesis suggests that the larvae carry the lectin while bacteria in the primary films produce the carbohydrate. Our preliminary results indicated that the cue for larval development of Janua is located in extracellular polymers of bacteria.

Our research during the past year has involved experiments designed to test whether the lectin model applies to invertebrates other than the spirorbid polychaete Janua. A clear understanding of the biochemistry of larval lectins may provide a powerful new insight into the mechanisms of larval settlement and metamorphosis.

During the past year we have isolated and identified over 100 bacterial isolates collected from larvae of the marine fouling bryozoan Bugula neritina. This project was initiated in cooperation with R. Woollacott of Harvard University to characterize the bacteria known to be present within the larval pallial sinus. We are presently completing the identification of the bacteria associated with the external and the sinus surfaces of B. neritina.

Bryozoans appear to harbor unusual microorganisms. We are also examining larvae of Watersipora, which appear to contain a population of bacteria that lack cell walls. We intend to determine both the nature and function of these bacteria.

FUTURE PLANS

(a) Continuation of our experiments on the mechanisms of lectin-mediated settlement in selected marine invertebrates; (b) studies on the substances, produced by bacteria, that induce marine invertebrate larval settlement and metamorphosis; (c) continuation of our detailed research on lectin-mediated larval settlement systems; and (d) continued analysis of the bacteria that are associated with marine fouling and boring organisms. Studies on the bacteria associated with the pallial sinus of specific species of bryozoan larvae will be continued, to determine the exact identity of the bacteria as well as the role that they play in the larval development process. We will continue the studies on the unusual bacteria that lack a cell wall found in close association with larvae of the bryozoan Watersipora sp..

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BIOCHEMICAL CONTROL OF MARINE FOULING

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OBJECTIVES

(a) To identify and characterize the surface-associated and ocean-borne biochemical substances, and the basic biochemical mechanisms, which control the recruitment, attachment, and metamorphosis of fouling-species larvae, and thus determine marine fouling; and (b) to identify those critical processes in the control of marine fouling which are most susceptible to useful biochemical blockade.

ABSTRACT

Marine invertebrate animals and plants which foul and encrust the submerged surfaces of ships, piers, pilings, caissons and lines are recruited to these surfaces as larvae from the plankton. In our recent biochemical research, we have shown that the recruitment of many important groups of fouling species is dependent upon detection, by the planktonic larvae, of specific biochemical signals or triggers which are required for induction of larval settlement, attachment, and metamorphosis. We have found that these naturally required biochemical inducers of fouling, in the case of a large number of molluscan and tube-building worm fouling species, comprise a family of macromolecular protein conjugates of structurally related, simple, amino acid-derived analogs of neurotransmitters, which are produced by algae, bacteria, or adults of the fouling species associated with the recruiting surfaces. Ocean-borne molecules structurally related to these surface-associated inducers of fouling also play a role in regulating and facilitating the fouling sequence.

In our most recent research, we have identified the basic and essential common biochemical and cellular elements of the fouling sequence which may be useful targets for the development of practical interdiction and control measures. These essential common elements, which we have found to control the recruitment, attachment, metamorphosis, and growth of both the attached mollusc, Haliotis, and the fouling, tube-building and cementing worm, Phragmatopoma, include: (a) Recognition, by the drifting, planktonic larvae, of surface-associated chemical inducers of fouling; (b) Induced efflux of specific ions, apparently moving across the chemosensory epithelial membranes of the recruited, fouling larvae, and found by us recently to act as essential transducers of the signal which triggers the fouling sequence; (c) Rapid synthesis of cyclic nucleotides, which act as essential intracellular transducers of the ion efflux from the signal-activated chemosensory membrane; (d) Secretion of specific glycoproteins(s) from specialized glands in the head of the fouling larva; these induced secretions apparently mediate the first steps in larval attachment and strong chemical and physical adhesion to the recruiting surface; (e) Loss (frequently by abscission or shedding) of specialized epithelial appendages

required for planktonic larval swimming, thus resulting in the irreversible commitment of the recruited larvae to the attached or surface-associated mode of life; (f) Internal organogenesis and metamorphosis of the recruited larvae to the benthic juvenile organisms; (g) Secretion of hard, protective shell or tube structures, or other protective encasements and/or permanent attachments; and (h) Rapid growth of the attached organisms.

Our studies now are focused on the critical early biochemical processes essential for the fouling sequence, to determine their sensitivities to practical means for biochemical or other new means for effective blockade and control. We have found that recognition of the surface-associated biochemical signals required to induce the fouling sequence is mediated by stereochemically specific chemosensory receptors located on specialized structures on the larval cephalic epithelium. Activity of these receptors is regulated both developmentally (internally and genetically), and "allosterically" by external, ocean-borne soluble organic molecules present in especially high concentrations in coastal waters. Generalized receptor-blockers, such as lectins, and blockers of the induced ion flux transducers (by weak opposing electrochemical gradients), thus far appear to be most widely useful for inhibition of fouling.

PLANS FOR THE FUTURE

(a) Further biochemical characterization of inducers, ocean-borne regulators, larval receptors, and ion-flux transducers of the fouling sequence in Haliotis, Phragmatopoma, and other species; (b) Characterization of sensitivities of these processes to biochemical and other new means of inhibition.

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PHYSICAL MEASUREMENTS OF WAVE-GENERATED FORCES ON A BIOLOGICALLY
SIGNIFICANT SCALE, AND THEIR ECOLOGICAL CONSEQUENCES

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OBJECTIVES

(a) To design and construct a telemetry system and recording dynamometers to measure the hydrodynamic forces imposed on intertidal fouling organisms by breaking waves, (b) to use these instruments to characterize the water flow during breaking waves and to measure the forces imposed on a variety of animals attached to rocky shores, (c) to use these data to examine the mechanical design and adhesive capabilities of fouling organisms, and (d) to re-examine the role of wave forces in structuring the fouling community.

ABSTRACT

Wave forces have long been thought to serve as a controlling factor in the structure of intertidal fouling communities, however, quantitative information regarding the forces imposed on fouling organisms by breaking waves and the ability of organisms to withstand these forces is nearly non-existent. To remedy this situation we have designed a simple FM/FM telemetry system capable of measuring the directional components of the forces imposed on small animals (e.g., barnacles and limpets) by individual waves. The apparatus has been used at two sites on Tatoosh Island, near Cape Flattery, Washington; both sites being fully exposed to the prevailing Pacific swells. A total of approximately 100 hours of force recordings have been obtained for two species (an acorn barnacle, Semibalanus cariosus, and a limpet, Collisella pelta) and for small plastic spheres used to calibrate and standardize the system. The adhesive tenacities of six common intertidal species have been measured. These data have been partially analyzed and a preliminary picture of the results can be drawn.

(a) Water velocities and accelerations at the rock surface are very high. The maximum values measured (with a wave height of approximately 4 m at breaking) were 12 m/s and 504 m/s². It can be estimated that during extreme storm conditions (wave height at breaking = 10 m) flow values of 14 m/s and greater than 1000 m/s² may be reached.

(b) These flow conditions place considerable forces on fouling organisms adhering to the rock surface. Each species exhibits a distribution of adhesive tenacities and the probability that an organism may be dislodged during specific wave conditions can be calculated. For organisms with large tenacities and small drag, lift, and added mass coefficients this probability may be relatively small; 15% for the acorn barnacle Balanus glandula (at flow conditions of 14 m/s

and 1000 m/s^2) while for other species with smaller tenacities and less streamlined shapes the probability of dislodgement is high; 72% for a solitary mussel, Mytilus californianus.

(c) The stress (force/area) imposed by breaking waves on the adhesive system of intertidal organisms is, in part, a function of the size of the organism; the larger the animal the greater the stress and the greater the adhesive tenacity required to resist dislodgement. This dependence of stress on size is a result of the rapid water acceleration occurring during wave breaking, and for those species with an appreciable probability of dislodgement this factor may serve to limit the size to which these organisms can productively grow.

(d) Surprisingly, substantial lift forces (ie. forces perpendicular to and away from the rock surface) are encountered by intertidal organisms during rapid water flow. For organisms with relatively large lift coefficients (C_L) (ie. the limpet Collisella pelta, $C_L = 0.33$ at a Reynolds number of $1 \cdot 10^5$) the lift force exceeds the drag force. To a certain extent C_L can only be minimized by maximization of the drag coefficient, and the evolutionary design of shell shape in intertidal organisms may represent an attempted compromise between lift and drag forces.

(e) A negative correlation was found between wave force and the frequency with which that force was encountered. However no correlation was found between still water level and the time at which the maximum force was encountered during a particular tide; ie. an animal may be washed-over by fewer waves at low tide than high tide, but the probability of being hit by the maximum wave force is independent of the water level. These factors may influence the foraging strategies of mobile fouling organisms.

PLANS FOR THE FUTURE

This is the final year of this contract. Data analysis will be completed and publications prepared. Further work is planned on the design of the adhesive systems of intertidal organisms, on the relationship between lift and drag coefficients, and on the effect of microhabitat on wave forces encountered

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- (c) M.W. Denny. "Wave forces on solitary intertidal organisms." in preparation.
- (d) M.W. Denny. "A simple recording dynamometer for measuring wave forces on intertidal organisms." in preparation.
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Effect of molecular-microfouling on macrofouling settling
variability

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OBJECTIVES

To define significant properties of molecular and microfouling which are influential in attracting subsequent macrofouling growth by a) establishing the rate of attachment of microorganisms (bacteria and diatoms) and macrofouling larvae (bryozoans) on substrates treated with different molecular weights of seawater and b) determining the degree of variability in numbers and distribution of macrofouling larvae.

ABSTRACT

Prior history of substrates has been established as a critical factor in the subsequent development of macrofouling organisms with molecular organic films, bacteria and diatoms all being implicated in conditioning the substrates. Preliminary experiments indicated that a rod-shaped bacterium isolated from nearshore waters in Puget Sound attached differentially within the same time frame to glass slides coated with various organic fractions. The organic-rich fraction caused a reduced attachment of bacteria in the time during which greatest attachment occurred in other fractions. Some cells which appeared irreversibly attached at 20 minutes in the low-organic coatings subsequently detached. Glass slides immersed in the bacterial suspension for 50 minutes next received additions of the diatom Amphora coffeaeformis. Although there was not sufficient opportunity to repeat the experiments, analysis of variance indicated some statistically significant interactions between treatments.

Current experimental procedures have included a) separating seawater into high and low molecular weight fractions, b) obtaining bacterial cultures from nature and culture collections and maintaining them in the laboratory, c) obtaining and maintaining diatoms, especially Amphora coffeaeformis, a benthic species known to settle, in cultures and growing them in relatively large quantities; d) obtaining bryozoans, primarily Bugula pacifica, from the field, maintaining them in the laboratory and inducing release of their larvae.

Fouling experiments with glass slides as a substrate have been conducted using various combinations of bacteria only, bacteria plus diatoms, bacteria plus larvae, diatoms only, diatoms plus larvae, bacteria plus diatoms plus larvae, and larvae only. Preliminary experiments with rough formica as a substrate have been done using larvae only.

PLANS FOR FUTURE

A) To establish the rate of attachment of microorganisms and macrofouling larvae on surfaces as a function of preceding chemical and biological treatment and to determine the degree of variability in numbers and distribution of macrofouling tunicates and polychaete larvae separately, B) to establish the degree of variability in the number of macrofouling larvae settling on surfaces as a function of preceding chemical and biological treatments involving combinations of microfouling species.

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PROGRESS REPORT ABSTRACTS OCEANIC BIOLOGY PROGRAM(U)
OFFICE OF NAVAL RESEARCH NSTL STATION MS DEC 82

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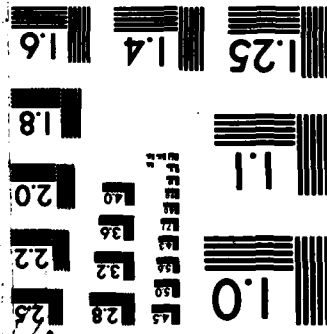
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MICROCOPY RESOLUTION TEST CHART
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MOLECULAR BASIS OF MARINE FOULING

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OBJECTIVES

The ultimate goal of our research is to understand the factors that are involved in marine microbial adhesion to surfaces and to develop approaches that could be used to control this process. The work involves (a) developing genetic tools to analyze microbial adhesion and behavior, (b) isolating and characterizing the genes that encode these properties, and (c) manipulating the systems that provide the bacteria with the ability to interact with a large variety of surfaces.

ABSTRACT

In order to develop appropriate genetic tools, we began our work with a relatively well characterized property, bioluminescence in a ubiquitous group of marine bacteria the vibrios. We have thus far succeeded in:

- (1) preparing gene banks from a variety of marine bacteria including Vibrio harveyi, V. fischeri, and V. parahaemolyticus.
- (2) preparing oligonucleotide probes and using them to isolate the α and β luciferase genes from Vibrio harveyi;
- (3) preparing mutants using transposons and using these mutants to identify the genes for bioluminescence;
- (4) transferring the bioluminescence system into E. coli where gene regulation can be studied;
- (5) using a variety of transposon systems to identify the other genes involved in bioluminescence including, aldehyde genes, regulatory genes and structural genes;
- (6) analyzing the plasmids carried by a variety of marine vibrios;
- (7) preparing antibody to various cell surface antigens purified from the Vibrios.

PLANS FOR THE FUTURE

We have gained sufficient experience with this system so that we can now directly approach the problems of adhesion. This approach will take a number of forms. We plan to isolate from the transposon-carrying cells, mutants that are defective in adhesion. We are looking both for mutants that show the inability to adhere efficiently to a variety of test surfaces and for mutants that are defective in specific cell surface properties. We will test a number of hypotheses that have been invoked to explain adhesion, including: (a) the notion that flagellar organelles and swarming cells are important (b) that the formation of specific cell surface polysaccharides is an integral part of the adhesion process.

We will identify specific genes and gene products that are affected in the adhesion negative mutants.

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3. Melvin Simon, Michael Silverman, Robert Belas, Alan Mileham, John Abelson, Richard Ogden and Dan Cohn. Marine Fouling. 10th Annual MIT Sea Grant Symposium, Biotechnology in the Marine Sciences, Cambridge, Massachusetts, submitted for publication (1983).
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THE ROLE OF EXOPOLYMER BINDING IN MICROFOULING AND SEDIMENT ERODABILITY

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N00014-82-C-0404

NR 083-231-10

OBJECTIVES

Our objectives are four-fold: (a) to improve the sensitivity and resolution of the gas chromatograph-mass spectrometer (GC/MS) analysis of uronic-acid components of exopolymers; (b) to determine the relationship between exopolymer and the microfouling film cells as it relates to heat-transfer resistance in condenser simulators; (c) to develop methods for documenting the presence and activity of anaerobic bacteria in inducing corrosion in the microfouling film; and (d) to relate exopolymers concentration to sediment erodability.

ABSTRACT

We have pursued the development of a better assay of the uronic acids in exopolymers. The uronic acid components are found in polymers outside the cytoplasmic membranes of cells, and they have proved extraordinarily difficult to analyze. Quantitatively forming the methyl esters of the uronic acids while still in the polymer and then reducing them to the corresponding alcohols with sodium borodeuteride makes the polymers susceptible to acid hydrolysis. Each component carbohydrate can be isolated by gas chromatography (GLC) and the proportion of that sugar that was a uronic acid in the original polymer determined. We have found that by forming the nitriles of the component aldose sugars after hydrolysis, we achieve better resolution, save a time-consuming step, and improve the sensitivity.

Why go to all this trouble? The uronic acid-containing exopolymers are thought to be of critical importance in the adhesion of bacteria. We have studied exopolymer formation and composition in two areas of importance to the Navy: the irreversible steps by which microbes form the initial microfouling film and the mechanism by which microbes affect the erodability of sediments.

We have established, at least for one marine microorganism, that both total exopolymer production and the proportion of the polymer hexoses that are uronic acids increase with increasing metabolic stress to the organism. This established for the first time that a single bacterial monoculture could change the composition of the exopolymers formed during the growth cycle. This has allowed us to produce varying exopolymer concentrations and compositions in defined sediments with which we were able to show that the critical erosion velocity of sediments is proportional to the galacturonic acid content of the sedimentary exopolymer. We have also been able to show that in the microfouling film from brushed metallic surfaces exposed to running seawater the presence of galacturonic acid in exopolymer has a much more significant effect on the resistance to heat transfer than the total

biofilm cellular biomass.

We have under development a sensitive assay for the presence of anaerobic bacteria in these biofilms. This is an important measurement because some of the mechanisms of microbially facilitated corrosion in metals exposed to seawater depend upon the generation of reduced compounds such as hydrogen, hydrogen sulfide, and organic acids, all of which involve the activity of anaerobic bacteria in microfouling film.

PLANS FOR THE FUTURE

With our assays for the uronic-acid components of the exopolymer and the biochemical measures of microbial biomass and community structure, we plan to examine in detail the role of the exopolymer in the initial steps of bacterial adhesion and to continue studies of exopolymer effects on sediment erodability.

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BIOLOGICAL STUDIES OF MARINE BORING AND FOULING MOLLUSKS

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NR 104-687

OBJECTIVES

As in past years the objectives of this long term project have been concerned with: a) the role of wood borers in littoral (Teredinidae and Martesinae) and deep-sea (Xylophaginae) ecosystems, b) life history studies of shallow water forms and the application of these studies to the ecology and classification of the species, c) the role of bacteria (symbiotic and/or commensal) in the nutrition of wood borers, d) distributional studies, particularly in areas where the activities of these organisms conflict with man's interests.

ABSTRACT

During the past year emphasis was placed on filling data gaps in our life history and bacterial studies (Objectives b and c). Field work in Venezuela (in cooperation with Prof. J. Ewald, Zulia University, Maracaibo) confirmed our belief that Teredo fulleri Clapp was a long-term sequential brooder. Other brooders found with fulleri in a rather exposed mangrove area (salinity 35-40 ‰) included short-term brooders in both Lyrodus and Teredo. Though brooders are not generally found in mangrove their occurrence at this site does not conflict with the hypothesis presented last year that brooders occur only where circulation is good, the water clear and oxygen tension high. Further work on the Sarawak survey (in cooperation with Siang Kok, Director of Forests, Kuching) continues to support this theory.

The Venezuelan survey also showed that Psiloteredo healdi (Bartsch), the only species of shipworm living in Lake Maracaibo south of the city, can tolerate salinities ranging from 4 to 20 ‰. A collecting panel containing P. healdi removed from a test site in the lake near Maracaibo was hand carried to Cambridge and maintained at a salinity of 7 ‰ (the salinity at the site at the time of removal). Specimens removed from the panel were used (in cooperation with Dr. John Waterbury, Microbiologist, WHOI) to further our study of the role of symbiotic bacteria found in the Gland of Deshayes in the nutrition of teredinids. The bacteria cultured from this tropical brackish water species appear to be the same as that taken from species of Bankia, Teredo, and Lyrodus from cooler, fully saline waters. This suggests that the same bacterium is found throughout the Teredinidae. Though P. healdi is often found living in very low salinities, bacterial cultures from this species grew much better when the salinity was raised to 15 ‰. This further suggests that the bacteria probably have a narrower range of salinity tolerance than the shipworm, and that shipworms living at low ambient salinities may not be able to control the salinity in the Gland of Deshayes. If this is so then the need to maintain bacteria in the Gland of Deshayes may be an important factor in preventing the invasion of freshwater by shipworms. Field and laboratory experiments are planned to test this theory. In addition we plan to look at the Gland of Deshayes in other genera.

Continuing developmental studies have shown that the Teredinidae, unlike most bivalve lineages which contain brooding species, are not characterized by a trend toward increased lecithotrophy among the brooding species. The reproductive type of a teredinid therefore cannot be inferred from the well documented correlation of bivalve egg size, larval shell size and morphology, and developmental type. Thus the larviparous teredinids cannot be placed in the general classification of bivalve developmental types. However, within family comparisons of larval shells representing the main teredinid reproductive patterns show that the developmental type can be determined by an analysis of straight-hinge and pediveliger shell size.

No deep-sea stations were visited this year but work on panels retrieved earlier continues to produce interesting data. A wood panel (65 X 15.5 X 2.5 cm) submerged for 2 years in 4000 m north of St. Croix produced 2048 specimens representing 9 phyla 35 families, and 81 species. More than 12,000 polychaetes have been sorted into 25 families, the most common being the Hesionidae (31%), Chrysotyalidae (13%), and the Phyllodocidae (9%). Two new genera and species of Ampharetidae have been described. Though the fauna of the 4 Atlantic bottom stations differ somewhat, the general diversity (number of species and number of specimens) and patterns of development are similar. For example, a good growth of hydroids always heralded the appearance of nudibranchs which feed on them. St. Croix at 4000 m is the deepest of our stations but was the most rapidly populated, probably because of its proximity to a forested island and the consequent abundance of wood on the bottom before the 'wood island' was put in place.

PLANS FOR THE FUTURE

During the ensuing year we plan to: a) complete described as in progress, b) look at the utilization of wood by Xylophaga atlantica Richards, the species found in deep set lobster pots on Georges Banks, to determine whether or not they have bacteria similar to those found in teredinids, c) conduct experiments to test our hypothesis concerning the absence of shipworms in freshwater, d) continue work on the 'Suevey and catalogue of the Pholadidae' and the 'Monograph of the Xylophaginae'.

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PROXIMATE INFLUENCES ON SETTLEMENT SUCCESS AND SPATIAL PATTERN IN INTERTIDAL BARNACLES

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NR. 104-179/5-28-82 420

OBJECTIVES

(a) To study patterns of daily settlement and survival of intertidal barnacle larvae under field conditions, (b) to determine the environmental correlates of success and failure in recruitment of barnacle larvae in the intertidal zone, (c) to determine how much of the microspatial variation in recruitment in natural populations is attributable to microtopography, and how much to chemical attractants associated with gregarious settlement.

ABSTRACT

A method has been developed to follow the fates of individual larvae that settle on each day in the settlement season. Permanent quadrats (6 cm x 9 cm) in the rocky intertidal zone were used to document settlement and survival. The quadrats were marked with stainless steel screws set in wall anchors in holes drilled in the rock. A focal frame on a 35 mm camera fitted in the slots of the screws, providing precise registration of camera position from photograph to photograph. Enlargements (20 cm x 24 cm) of the negatives are digitized with a graphics tablet interfaced with a minicomputer. The coordinates of each individual animal on each enlargement are digitized. The coordinate systems of all photographs are scaled identically by digitizing a ruler in each photograph. The coordinate systems of sequential photos of the same site are translated and rotated until they superimpose (least squares fit) upon the coordinate system of the first photograph in the series. The coordinates of each individual and associated life stage (cyprid larva, uncalcified metamorphosed barnacle, calcified metamorphosed barnacle) to identify all individuals on successive photographs, as to the daily cohort to which they belong. Daily cohort survivorship curves are then constructed. Three years of existing daily settlement photos from natural rocky intertidal sites at Nahant Massachusetts, will be analyzed in this study.

PLANS FOR FUTURE

In order to determine the environmental correlates of success and failure in recruitment of barnacle larvae in the intertidal zone, a statistical model will be developed of survival in relation to temperature, humidity, cloud cover, rainfall, wind and time of low tide. A canonical correlation model will be constructed from the data for one year, and will be tested with data for the other two years.

In order to estimate the relative importance of microtopography and chemical cues in influencing the spatial pattern of settlement, the settlement patterns of larvae in the same site in different years will be compared. If intensity of settlement is related to the proteins remaining on the rock after the death of the inhabitants of a site, then settlement should be concentrated in microsites where adults were living in the previous year. If microtopography has a dominant influence on settlement, then in one site settlement should occur in the same pits and grooves in each year.

This is a new project which started September 1, 1982.

STUDIES ON THE MOLLUSCAN WOOD BORER
BANKIA GOULDI

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N00014-78-C-0294

NR 104-194

OBJECTIVES

(a) To investigate growth of the marine wood borer Bankia gouldi as indicated by rates of boring, protein synthesis, and CaCO_3 deposition; (b) to define the major processes involved in formation of calcified tubes of Bankia; and (c) to examine conditions which influence in vitro mineralization of decalcified skeletal material of fouling organisms and crystal formation in metastable CaCO_3 solutions.

ABSTRACT

Bankia removed from the wood, stripped of their calcified burrow linings, and placed in sea water will form a calcified tube similar to the natural burrow lining, as reported earlier.

Deposition of calcium carbonate crystals is associated with organic material, and alternating layers of prismatic and granular structures resembling similar structures in molluscan shell are formed. Organic matrix of burrow lining decalcified in the presence of fixative to prevent the loss of soluble organic material can be partially recalcified in solutions with an ionic composition similar to molluscan extrapallial fluid. This demonstrates the capacity of the matrix material to initiate mineralization even though altered by fixation. However, examination of the recrystallized material by scanning electron microscopy has shown that the recalcified mineral structure is not similar to the mineral layers prior to decalcification.

We have investigated the possible influence of the extrapallial fluid and insoluble shell matrix of molluscs (Crassostrea virginica and Mercenaria mercenaria) on the rate of calcium carbonate crystallization in a metastable solution similar in ionic composition to extrapallial fluid. Both the extrapallial fluid and the insoluble shell matrix were strongly inhibitory in high dilution. Previously, both had been considered as possible initiators of crystal formation but evidence was lacking. Our previous work indicates that the inhibition by extrapallial fluid on crystal formation may be due to binding of acidic protein residues on the crystal surface.

PLANS FOR THE FUTURE

Research under this contract was terminated in February 1982.

CURRENT REPORTS AND PUBLICATIONS

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LIFE-HISTORY STUDIES OF FOULING BRYOZOANS

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N00014-78-C-0064

OBJECTIVES

Our objective is to build an understanding of the fundamental events and processes in the larval biology, settlement, and metamorphosis of major species of fouling bryozoans. Special emphasis is placed on: (a) mechanisms of phototaxis, geotaxis, and competency for metamorphosis; (b) bacterial films and larval settlement; (c) analysis of rapid morphogenetic movements in metamorphosis and their chemical inhibition.

ABSTRACT

In spite of the importance of bryozoans as significant components of fouling communities, little is known about their reproduction and, consequently, of how infestations are established. Our research focuses on the *Ce lularioidea*, a superfamily of anascan cheilostomes, that contains genera (e.g., *Bugula*, *Tricellaria*, and *Scrupocellaria*) with numerous world-wide fouling species.

First, field studies continued this year on the occurrence of bacteria in certain species of *Bugula*, continuing 13 years of data on California species and 4 years on Woods Hole species.

Second, work in collaboration with Drs. Paul Boyle and Ralph Mitchell (Harvard University) resulted in isolation of the *B. neritina* symbiont and those of two other bryozoan species. The *B. neritina* symbiont has now been characterized and identified.

Third, laboratory experiments were concluded on the preference for filmed versus unfilmed surfaces in three sympatric congeners. Preliminary investigations were initiated on the specific properties of microbial films recognized by bryozoan larvae.

Fourth, experimental and morphological analysis of preancestrula formation in *B. neritina* were completed. These studies focus on the role of microfilaments in morphogenetic movements.

PLANS FOR FUTURE

(a) Continued collaborative efforts with Ralph Mitchell's laboratory to identify the transported bacteria and to assess its potential role in the settlement process; (b) completion of analysis of geotaxis by *Bugula* larvae; (c) continuation of investigation into properties of films that trigger settlement; (d) analysis of mechanisms of transport of bacteria from generation to generation of bryozoans.

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- (b) Reed, C.G. and R.A. Cloney. 1982. "The larval morphology of the marine bryozoan Bowerbankia gracilis (Ctenostomata: Vesicularioidea)". Zoomorphology, 100, pp 23-54.
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- (d) Brancato, M.S. and R.M. Woollacott. In press. "Effect of microbial films on larval settlement of three species of Bugula (Bryozoa)". Marine Biology.
- (e) Reed, C.G. and R.A. Cloney. In press. "Metamorphosis of the marine bryozoan Bowerbankia gracilis (Ctenostomata: Vesicularioidea)". Zoomorphology.
- (f) Woollacott, R.M. In press. "Environmental factors in bryozoan settlement". In: Marine Biodeterioration and Fouling, J. Costlow and R. Tipper (Eds). U.S. Naval Academy Press, Annapolis.
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- (h) Pires, A. and R.M. Woollacott. Submitted. "A direct and active influence of gravity on the behavior of a marine invertebrate larva". Science.

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ADDENDUM

SOURCES AND DISTRIBUTIONS OF OCEANIC BIOLUMINESCENCE

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WORK UNIT NO. NR 083-165

CONTRACT N00014-81-C-0062

OBJECTIVES

(a) To identify the organisms responsible for near-surface bioluminescence in oceanic waters, (b) to assess their relative importance in nocturnal bioluminescence production, (c) to measure the vertical and horizontal patterns of near-surface bioluminescence, (d) to measure the color and intensity of the bioluminescence, (e) to understand factors leading to the vertical positioning of populations of bioluminescent species, (f) to understand how bioluminescence increases the survival of organisms in the natural environment.

ABSTRACT

We have completed the analysis of bathyphotometer data from five cruises to the Sargasso Sea and the Gulf Stream; R/V TRIDENT Cruise 177, C. O. ISELIN (October 1976), and R/V ENDEAVOR Cruises 049, 054 and 073.

We recorded vertical profiles of bioluminescence in the upper 200m using a pump-through bathyphotometer (4-liters min^{-1}). We have finished the identification of the organisms in $25\mu\text{m}$ porosity nets attached to the effluent of the bathyphotometer at selected depths. Analysis of the data suggests that the dinoflagellate *Pyrocystis noctiluca*, copepods, larvaceans, and ostracods are the major contributors to bioluminescence. In the regions with greater biomass, the Gulf Stream, the northern Sargasso Sea, passages between the Antilles Islands, there are pronounced peaks of bioluminescence potential at various depths. Generally, the maxima in bioluminescence potential are shallower than those for the chlorophyll maximum.

On cruises 054, 073 and 082 of R/V ENDEAVOR we determined the bioluminescence potential of dinoflagellates, crustaceans and larvaceans. Most of the tested organisms have been identified to species.

A number of experiments were run on the behavior of copepods in the presence of bioluminescent dinoflagellates. Simulated bioluminescent dinoflagellate flashes were shown to cause a "startled" reaction by the copepods.

PLANS FOR THE FUTURE

(a) To determine the organisms primarily responsible for bioluminescent displays in temperate and boreal oceanic waters, (b) to examine and to correlate patterns of bioluminescence potential with hydrographic and other factors, (c) to study the contribution to surface displays of large crustaceans and other invertebrates too agile to be captured by a pump-through bathyphotometer, (d) to

examine the behavior of bioluminescent organisms to understand the advantage it has for their survival in the sea.

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GUIDE TO SHARKS OF THE WORLD

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NO14-80-C-0282

104-168

OBJECTIVES

To prepare textual information and to select illustrated material for a Guide to Sharks of the World, which will include data on their identification, natural history, distribution, and human impact. The Guide will be in a three-hole, loose-leaf format, similar to the regional fisheries identification sheets published by the Food and Agriculture Organization of the United Nations for fishes and other marine animals.

ABSTRACT

The Guide to Sharks of the World includes identification sheets for all eight orders of living sharks, all 30 shark families, species sheets for about half (170) of the living species of sharks, and generic sheets for certain large families (Squalidae, Scylliorhinidae, Triakidae, and Carcharhinidae). The ordinal sheets include a common and scientific ordinal name, a short list of field marks for quick ordinal identification, a diagnosis listing the ordinal characters, similar orders, a list of families and key to families in the order, and a pictorial guide to the families in the order. The family sheets include a common and scientific familial name, a short list of field marks for quick family identification, a diagnosis listing the familial characters, color, size, behavior, feeding habits and reproduction, distribution and habitat, danger to humans, interest to fisheries, similar families, a list of species, keys to genera, genera and species, or species only, references in abbreviated form, and a pictorial guide to genera. Species sheets include family names, common and scientific names of the species, a lateral view, ventral head view, and sometimes representative teeth as illustrations, field marks, diagnosis, color, size, similar species, behavior, feeding habits and reproduction, distribution and habitat, danger to humans, interest to fisheries, a world map showing geographic distribution, and abbreviated references. The genus sheets, where needed, include family, common, and scientific names, an illustration in lateral view of a typical species, field marks, diagnosis, and optional space for additional data including keys to species.

Using the format for the Guide finalized in the last contract period, ordinal, family, and species sheets were prepared for the shark taxa to be included in the Guide, with considerable information to be added to most of the sheets. Work on the text of the Guide was interrupted by an ONR-U.S. State Department sponsored tour of selected foreign museums, field stations, and other research organizations for the express purpose of collecting data to be used in the Guide and related projects and for preparing illustrations of sharks. The tour lasted almost 6 months, from 12 June 1982 through 6 December 1982, and included as an itinerary Victoria University of Wellington and National Museum of New Zealand, Wellington (12-18 June); Australian Museum, Sydney (19-24 and 28-29 June); Commonwealth Scientific and Industrial Research Organization, Marine Laboratories, Cronulla, Australia (25-27 June); Western Australian Museum, Perth (29 June-5 July); U.S. Embassy, New Delhi, India (6-9 July and 31 July-11 August); Government of India, Central Marine Fisheries Research Institute, Cochin (headquarters and field station), Trivandrum (field station) and Tuticorin (field station; 9-22 July); Zoological Survey of India, Calcutta (22-31 July); Government of India, Departments of Science and Technology, Ocean Development, and Agriculture (Fisheries Division; 5 August); J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa (August 12-16 September, 19 September-17 October, 24 October-1 November); Port Elizabeth Oceanarium, South Africa (16-18 September); Oceanographic Research Institute, Durban (headquarters) and Richards Bay (field station), South Africa (17-24 October); and Fisheries Resources and Environment Division, Food and Agriculture Organization of the United Nations, Rome, Italy (2 November-6 December). Additional stops in the tour at Abu Dhabi, Somalia, Kenya, and Israel had to be cancelled en route due to various problems making it impossible or unnecessary to visit these places at the time. Data gathered on the tour and the length of the tour itself made it necessary to postpone completion of the written part of the Guide until 1983, to request an extension of the 1982 contract beyond the original closing date of December 31, 1982, and to request a contract renewal to complete the Guide.

PLANS FOR FUTURE

Completion of my written contributions to the Guide to Sharks of the World in 1983.

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(a) L.J.V. Compagno (1982). Galeomorph sharks. In S. Parker, ed., Synopsis and classification of living organisms. McGraw-Hill, 2 vol., 1232 pp.

(b) L.J.V. Compagno (in press, 1982, to be published 1983). FAO Sheets. Sharks. Fishing Area 51. Food and Agriculture Organization of the United Nations, Rome, about 200 pp.

(c) L.J.V. Compagno (manuscript submitted 1982, to be published in 1983). FAO Species Catalogue. Vol. IV. Sharks of the World. Food and Agriculture Organization of the United Nations, MS about 600 pp.

* Includes only items of special relevance to the Guide.

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